# Adaptations to changes in the acoustic scene of the echolocating bat

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# Abstract

Our natural environment is noisy and in order to navigate it successfully, we must filter out the important components so that we may guide our next steps. In analyzing our acoustic scene, one of the most common challenges is to segregate speech communication sounds from background noise; this process is not unique to humans. Echolocating bats emit high frequency biosonar signals and listen to echoes returning off objects in their environment. The sound wave they receive is a merging of echoes reflecting off target prey and other scattered objects, conspecific calls and echoes, and any naturally-occurring environmental noises. The bat is faced with the challenge of segregating this complex sound wave into the components of interest to adapt its flight and echolocation behavior in response to fast and dynamic environmental changes.

In this thesis, we employ two approaches to investigate the mechanisms that may aid the bat in analyzing its acoustic scene. First, we test the bat's adaptations to changes of controlled echo-acoustic flow patterns, similar to those it may encounter when flying along forest edges and among clutter. Our findings show that big brown bats adapt their flight paths in response to the intervals between echoes, and suggest that there is a limit to how close objects can be spaced, before the bat does not represent them as distinct any longer. Further, we consider how bats that use different echolocation signals may navigate similar environments, and provide evidence of species-specific flight and echolocation adaptations. Second, we research how temporal patterning of echolocation calls is affected during competitive foraging of paired bats in open and cluttered environments. Our findings show that "silent behavior", the ceasing of emitting echolocation calls, which had previously been proposed as a mechanism to avoid acoustic

interference, or to “eavesdrop” on another bat, may not be as common as has been reported.

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*"(...) How old I am, and yet how far from being what I should be. I shall from this day take the firm resolution to study with renewed assiduity and always to keep my attention well fixed on whatever I am about, and to strive every day to become more fit for what, if Heaven willst it, I am some day to be! (...)"*

*-Queen Victoria, 1837*

# Chapter 1

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# Scene Analysis

Every day, as I walk north on St. Paul Street toward campus, cars cross the street, birds chirp, pedestrians and their barking dogs pass me by, and a truck might be backing out of a side street, blasting its jarring warning-beeps into the morning. Each of these sounds is its own discrete event, but together they compose a scene comprised of both visual and auditory objects. As I move around my environment, these multi-sensory stimuli change and may be interrupted occasionally: sounds from different sources may overlap and visual objects may be partially occluded. Yet, I perceive a stable and continuous world. My brain appears to automatically and effortlessly process this mix of sensory inputs –visual, auditory, maybe even tactile and olfactory- and merges them into a coherent percept of my immediate surrounding that informs me about how I should place my next steps to get to my destination. Understanding the processes that help us distinguish these objects from one another can inform us about key components of scene perception (e.g. Bregman, 1990; Gibson, 1979; Shinn-Cunningham and Best, 2008).

The goal of this research is to investigate how continuously changing sensory inputs affect behavioral output. Specifically, I examine how changes of the acoustic scene affect flight and echolocation behavior in the big brown bat. To set the stage for this research, I will first give a brief introduction to visual and auditory scene analysis, and point out differences in the processes of each. With respect to visual scene analysis, I will

emphasize research on optic flow. Subsequently, I will introduce the big brown bat as the subject of studies in this thesis, and review literature pertinent to adaptations of echolocation and flight behavior. I will introduce the notion of echo flow, which relates loosely to the concept of optic flow discussed previously. Finally, I will give a brief description of each chapter in this thesis.

*A brief introduction to Visual and Auditory Scene Analysis*

In the scenario of walking down St. Paul Street there are at least two processes that have to occur in each sensory system. These processes are inherently different, because one aims to segregate the raw sensory input into components from the real world, while the other is related to our allocation of attentional resources to process and represent those components. For example, first, with respect to acoustic stimuli, the sound waveform that enters my ears at any given moment is a linear combination of different sound waves originating from different sources in my surroundings: the chirping bird, the beeping truck, the barking dog, etc. This unprocessed composite waveform by itself would be of little use to me in understanding my scene, so my brain forms the separate sound streams, or *auditory objects*, of "bird song", "truck noise" and "dog barks" from the mixture. Second, knowing the sources of all the auditory streams, is of little importance if I don't know which one I should be *attending* to: if I am about to cross the street, it would be more useful for me to attend to the truck's and traffic's noise, than listening to the bird song. In other words, I may direct my attention to one of the streams my brain formed, while ignoring the other ones. This process continues until I deem a

different stream to be of greater importance than that of my current state, and switch, for example, from attending to the truck's beeps to the dog's barking. These two components (forming auditory objects of a physically complex stimulus and allocating attentional resources to selectively process these stimuli) can be applied to the analysis of the same scene for optic, or maybe even tactile stimuli: extracting *visual objects*, and closely watching the traffic's movements while crossing the street (as opposed to watching birds in a tree), and extracting *haptic objects* and feeling the edge of the sidewalk as I step onto the smooth asphalt of the street, contribute to our complete analysis of the scene.

*Vision*

It follows, that scene analysis is not limited to unimodal sensory input. All sensory stimuli we receive contribute to the full percept of our environment. Commonly, scene analysis is studied in vision, often referred to as visual scene analysis, or VSA. Similar to our acoustic environment, our optic environment contains light reflections from different objects that the brain has to detect, discriminate and recognize. In all of these stages, attention can modulate the sensory information processing and shape perception.

The study of visual scene analysis was spurred by early research in humans on the gaze control in natural scenes showing that eye fixations were indeed not random, but concentrated on informative regions on the scene (Buswell, 1935). For decades after that, research on human visual perception has been concentrated on *parts* of scenes, rather than the visual scene as a whole (Henderson, 2003; Land, 2006). In recent years, much effort has gone into extending the field to more realistic situations such as natural visual

scenes (Henderson, 2003; Land, 2006; Võ and Henderson, 2010), and connecting the results from behavioral studies to different theoretical and computational models of scene analysis (e.g Wang, 2007) and visual perception (Bar, 2004; Itti et al., 2001; Wolfe, 1994).

To decipher features in a scene and group them into coherent objects, Gestalt principles are used. Gestalt principles describe rules based on which components in a scene can be discriminated, categorized, and used in further processing to establish the percept of the surrounding scene as a whole (Bar, 2004; Gibson, 1979; Kondo et al., 2017). Some grouping cues that are described as part of the Gestalt principles are *similarity* (objects of similar appearance are more likely to belong together), *proximity* (nearby components are more likely to belong together), *closure* (objects must have an outer contour), *good continuation* (components forming the smoothest contours group together), *good form* (contours forming "familiar" or "regular" and "simple" shapes belong together), and *bad form* (contour cues that indicate two components do not belong together). In short, each Gestalt principle reflects some subjective estimate that small portions of a larger scene ought to be grouped together. For example, when standing on a beach and looking out onto the ocean, small neighboring parts of the scene have the same darker shade of blue; we can group those together, based on their similarity, into the part of the scene that comprises the ocean. Even within the "ocean part" of the scene, we may group small neighboring parts of white color together and label them the "breaking waves". Other parts of the same larger scene may contain a different shade of blue, which we group together into the part of the scene that contains the sky, and so on.

Scene analysis is a complex, multiple-sensory problem that can be approached using different investigation techniques. Most of what has been discussed so far is descriptive of a scene in which one views stationary objects. However, in our daily lives we move within our environment, and the environment moves around us, creating dynamically changing and complex scenes. In VSA, research aims to understand how a changing scene influences behavior and future planning of movements. For the purpose of this thesis, I am now going to focus and elaborate on optic flow as an example of such a dynamic way that influences scene perception.

## *Optic flow*

Processing visual information is important to control locomotion among organisms that primarily use vision for navigation. For example, while walking down St. Paul Street, cars and trees pass me by – the cars in large part due to their own motion, the trees due to my own motion. This sensory input gives me regular updates about my location, my speed, and when I may be walking into something; I am able to parse the scene into rigid and moving objects. Importantly, in the absence of this visual information, it is nearly impossible to plan my future movements and adjustments of motion (e.g. speed) in order avoid obstacles.

### *Optic flow in humans*

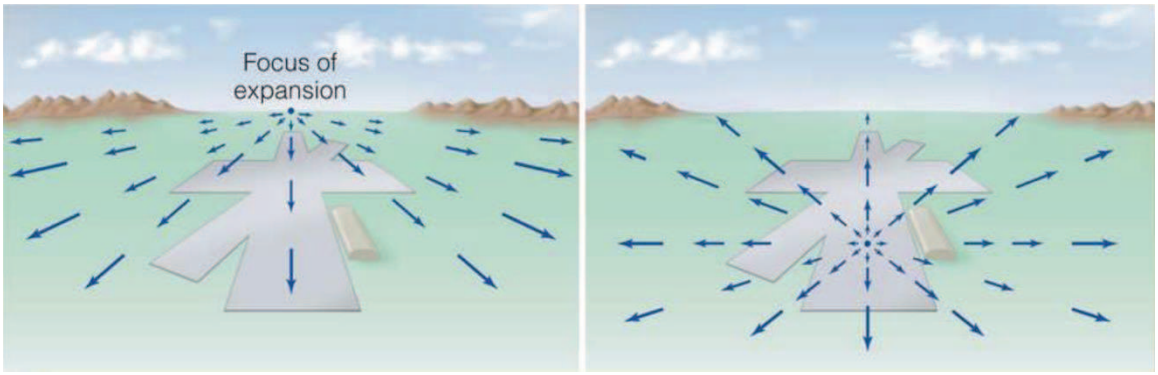
When we move through our environment, we experience ego-motion, which is fundamentally different than motion introduced by moving surrounding objects. We distinguish between the two by getting real-world spatial coordinates from our visual scene, feet and body movements during ego-motion, while those non-visual changes are

equal to zero when only objects around us move. Changes in the visual scene that occur from movement of oneself are commonly referred to as *optic flow (OF)*, a self-produced feedback of motion. In short, optic flow describes the dynamic features of objects as any observer moves past them (e.g. Gibson, 1950). It has also been described as the pattern of motion in the eye that specifies the direction of locomotion, thus the change of activation patterns across the retina (e.g. Srinivasan et al., 1996). Optic flow is processed continuously in our visual system and can be used to solve various tasks: it can support the estimate of self-motion or time-to-contact (e.g. Srinivasan et al., 1996), help us maintain balance (e.g. Prokop et al., 1997), and help to segment the scene into independently moving objects, foreground and background (e.g. Land, 2006).

James J. Gibson was a strong advocate for more natural experiments in vision research. He criticized that subjects could not move their heads during typical experiments, and that more often than not, research was investigating the study of "snapshot vision", rather than perception of a whole scene. He emphasized that the relationship between perception and movement is key to the understanding of environmental cues we use to inform our perception of the world. Motivated by wanting to provide an explanation of how pilots use environmental information to land airplanes, Gibson proposed two cues that observers use as they move through the environment: (1) the *gradient of flow*, which is the difference of flow percept as a function of distance from the observer, and (2) the *focus of expansion (FOE)*, describing a destination point in the distance where no flow is detectable (Figure 1). Gibson argued that an observer knows they are traveling in the correct direction by keeping the FOE in the direction they have to go to arrive at the destination. In other words, one would move such that the error

between where one is heading as perceived from optic flow and the actual destination is canceled out, which would continuously place the FOE on the target destination.

**Figure 1.** 1 **Schematic illustrating OF cues proposed by James J. Gibson (1950).**




Left: the focus of expansion (FOE) is the far point in the distance at which there is no flow percept. The length of arrows indicates flow speed – flow is greater in the foreground than the background (cf. motion parallax). The example illustrates a plane passing over a landing strip. Right: the gradient of flow is the change in flow percept as the observer gets closer to a specific destination point (FOE, blue point). The length of arrows indicates flow speed – speed is greater closest to observer. Here, the example illustrates a pilot landing a plane on a specific destination point on the landing strip.

Later experiments confirmed that humans do use optical flow information to navigate. Land and Lee (1994) asked drivers to drive a car along a winding road and measured the movement of the steering wheel time-locked to eye and head movements (Land and Lee, 1994). The results show that when driving along a straight path, drivers look straight ahead, though not necessarily at the FOE. In contrast, drivers make use of the gradient of flow when driving along a curved path, as shown by all subjects fixating on the inner edge of the road about 1-2 seconds before entering the curve. These results suggest that human drivers might be using other strategies in additional to optic flow to determine their heading direction. A few years later, Warren and colleagues (2001) added support to this finding by showing that humans walking on a treadmill while presented with different types of optic flow stimuli, guide their navigation using a combination of

optic flow cues and egocentric direction-taking, in which an observer perceives the direction of a goal with respect to their body and walks in that direction (Warren et al., 2001). They also show that humans rely on the same egocentric direction when no flow is available.

*Optic flow in non-human animals*

Most commonly, however, optic flow has been studied in small insects and birds (e.g. Srinivasan et al., 1996; Bhagavatula et al., 2011; David, 1982). Contrary to many other visually-guided animals, bees do not have stereoscopic vision and can thus not use it to their advantage to measure distances. It is surprising then, that when a bee flies through a hole in a wall, it flies through the center of that hole, balancing itself between the borders on each side. Such behaviors first motivated the study of the *centering response* in bees. As is outlined below, this research has shown that the centering response helps organisms navigate through tight spaces irrespective of the spatial structure of that space, and is driven mostly by a mechanism that is sensitive primarily to optic flow and speed estimation.

To test the effect of optic flow on the centering response, an animal will typically fly through a corridor, which spans some distance between a hive and a feeder. The corridor's walls will have different visual patterns printed on them. These patterns produce different strengths of optic flow across the animal's eye, and manipulation of the patterns allows for measurements of the impact that OF cues have on flight behavior. Overall, the most commonly studied patterns consist of black and white vertical and horizontal stripes or random check patterns. In some experiments, the thickness of the black and white stripes (or spatial frequency) is varied, in other experiments the stripe

patterns can move along the corridor in the direction of locomotion, or against it. Because optic flow increases with increasing speed of the observer and decreases with increasing distance from the pattern or row of objects, this experimental design allows the researcher to test a variety of hypotheses about the behavioral adaptations and perception of OF.

In the simplest case, an animal navigates through a series of corridor setups that provide it with either balanced or imbalanced OF cues. In baseline conditions, OF cues are the same on both corridor walls (balanced), whereas in experimental conditions the cues on each wall differ, providing the animal with different amounts of OF (imbalanced). In the former, the corridor can have vertical or horizontal stripes along both corridor walls, or also be evenly grey, a common control condition. In the latter, one corridor wall is lined with vertical stripes, which provide a strong OF cue, while the other side is covered in horizontal stripes, which provide a reduced or no OF cue. This basic setup has been studied in a variety of animals including honey bees (e.g. Srinivasan et al., 1991), bumblebees (Baird et al., 2010), drosophila (David, 1982), budgerigars (Bhagavatula et al., 2011) and even zebrafish (Scholtyssek et al., 2014). Researchers record the navigational paths while animals travel through the corridor and later measure the deviation from the midline between the two corridor walls, as well as flight speed.

Typically, research has established that animals center their flight paths in conditions of balanced OF cues, and deviate toward the side of weaker OF cues in imbalanced conditions (Baird et al., 2005, 2010; Bhagavatula et al., 2011; David, 1982; Kirchner and Srinivasan, 1989; Srinivasan et al., 1996, 1991). This behavior points towards the hypothesis that these animals are aiming to balance the optic flow across their two eyes: as they are distancing themselves from the wall with strong optic flow,

they are aiming to reduce that strong OF percept to be equal to the optic flow percept in the eye that is looking at the weak cue.

The optic flow balancing hypothesis was first confirmed in early OF experiments by Kirchner and Srinivasan (1989). Honeybees flew through different manipulations of OF corridors that had different gratings of black and white vertical stripes along both corridor walls. Overall, the centering response was not affected by exposing the bees to imbalanced vertical OF patterns (in this case different spatial frequencies of vertical stripes on either wall). But when one of the walls was moved at a constant speed in the direction of the bee's flight, the flight path shifted towards the moving wall. When the wall was moved in the opposite direction of the bee's flight, the bee's trajectory moved away from the moving wall. In the former case, the movement of the wall reduces the speed of retinal image motion in the eye viewing the moving pattern relative to the other eye, whereas in the latter case, the retinal image motion is increased. In other words, if the bee perceived fast image motion (or strong OF cue) in one eye and slow image motion (or a weak OF cue) in the other, it chose to balance the percept by deviating towards the slower image motion, thereby speeding it up, and deviating away from the fast image motion, thereby slowing it down. Srinivasan went on to show that this behavior is independent of the spatial structure of the stimulus, including whether patterns are sinusoidal gratings, or square-wave gratings (Srinivasan et al., 1991, 1996). He also showed that bees adapt their flight speed to keep the angular velocity of image motion, or the optic flow percept, constant – again, irrespective of the spatial structure of the pattern (Srinivasan et al., 1996). Baird et al., (2005) elaborate on this finding and argue that reducing the flight speed might help bees navigate in cluttered environments in

order to avoid object collision. In fact, when bees were trained to fly through a narrow (15 cm width) tunnel, they did so at lower speeds than flying through a wider (30 cm width) tunnel, which provided less of a collision-prone environment (Baird et al., 2010). Serres and colleagues extended this finding by using a 95 cm wide tunnel, mimicking open field navigation for the bee (Serres et al., 2008). Results of this study show that bees fly along the midline if both the feeder and hive of the bee are located around the midline, but they will shift their position laterally, if the feeder and hive are shifted. This finding suggests that optic flow cues can be disregarded in situations where potential collision objects are far away.

Recently, Dyhr and Higgins (2010) elaborated on earlier results of bees navigating through environments of different vertical gratings, as only select widths of stripes had previously been tested. In their study, they compared the flight patterns of bumblebees through corridors that contained a constant (grey) wall, and a wall with stripes of different spatial frequencies. Their in-depth analysis of the effect of low to high spatial frequencies shows that there is a decrease in deviation from the midline as the spatial frequency increases along the corridor walls. In other words, when the mismatch of the spatial frequencies on both corridor walls is large, bumblebees deviate toward the wall with lower spatial frequencies. However, as the spatial frequency increases, this bias gradually disappears such that at high spatial frequencies, bumblebees fly along the midline of the corridor. Dyhr and Higgins (2010) mention the possibility that this could be due to the fact that the bee may not be able to resolve the different gratings at high spatial frequencies and perceive them as a uniform gray wall. This is a point that I will discuss with regard to my own data in Chapter 7.

While all of the different species mentioned above center their flight paths between the two corridor walls when the pattern of optic flow cues is balanced, zebrafish are to-date the only species that has been shown to deviate *toward* the corridor walls lined with vertical black and white stripes in imbalanced conditions (Scholtyssek et al., 2014). Interestingly, zebrafish also do not adjust their velocity in response to different optic flow cues (Scholtyssek et al., 2014), indicating that (1) zebrafish do not control velocity by keeping the rate of optic flow constant or (2) balance the optic flow received across their two eyes. Scholtyssek et al. (2014) propose that this preference may point to an adaptation for locomotion control in murky waters.

To understand how visual animals might react to optic flow manipulations under low light conditions, Baird and colleagues tested how nocturnal bees navigated tight spaces at dusk and dawn (Baird et al., 2011). In contrast to bumblebees, the nocturnal bee *Megalopta genalis* does not adjust its flight trajectory to random or horizontal optic flow cues, though it does increase velocity with reduced OF cues (Baird et al., 2011).

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I will now introduce scene perception of acoustic stimuli. In this capacity, I will discuss some differences between visual and auditory scene analysis, and expand on cues that influence auditory scene analysis.

### *Auditory Scene Perception*

Similar to limitations in early behavioral VSA research, most early auditory scene analysis (ASA) research constrained the subject in their natural behaviors and asked them

to distinguish between different frequencies of pure tones, rather than natural sounds (e.g. Cherry, 1953). However, in any natural situation the soundscape is a cacophony of complex sounds. Fortunately, in recent years, research on auditory scene perception has incorporated more natural stimuli, such as simulated cocktail parties and recordings of real environments (e.g. Carlin and Elhilali, 2013; Carlyon et al., 2001; Hulse, 2002; McDermott, 2009). A large proportion of research now attempts to understand the role that attention plays in both visual and auditory scene analysis, and studies now oftentimes include EEG experiments in order to understand some of the potential underlying brain regions and neural components involved in ASA (Bronkhorst, 2000; Carlyon, 2004; Lee et al., 2014; O'Sullivan et al., 2015).

Central to the study of auditory scene analysis is the concept of "auditory streams" (Bregman, 1978). Auditory streams are formed as a result of successful segregation based on simultaneous and sequential grouping of acoustic cues. In simultaneous grouping, which occurs instantaneously, the timing of individual frequency components acts as an indicator for stream segregation (e.g. on- and offsets of a set of harmonics in a vowel, Darwin and Sutherland, 1984). Subsequently, these frequency components get grouped across time in a process that Bregman describes as "sequential grouping." Here, spectral components are grouped *over time*, which ensures that successive sound events from the same sound source are grouped together correctly into one auditory stream. In deciding whether two frequency components should be grouped together into the same stream, the auditory system makes use of whether sounds follow some pattern – a "good continuation," if you will, a Gestalt principle that is also used in visual scene analysis (see above). In other words, the auditory system will group

frequency components together that change slowly and coherently over time. Components which show sufficient variation from one instant to the next are less likely to be included in a successful stream (van Noorden, 1975). Auditory stream formation, thus, is rarely instantaneous. According to Al Bregman (1990), the auditory system gathers information for perceptual organization of sounds, a process which takes some time, normally on the order of a few seconds (Anstis and Saida, 1985).

*Comparing Visual and Auditory Components of Scene Analysis*

Our visual environment, much like our auditory surroundings, contains different objects. However, there are profound differences in the physical optical and acoustical stimuli, which give rise to visual and auditory percepts, and in the way that they are processed and grouped into coherent objects. In vision, as well as in audition, the object we focus on and attend to is impacted by a combination of factors like its saliency, which is influenced by physical stimulus dimensions (e.g. brightness, or size for vision; intensity or frequency in audition), but also the goals of the observer, the task on hand or behavioral states (e.g. Desimone and Duncan, 1995; Carlyon, 2004). Visual objects may occlude one another. In such cases, Gestalt principles aide in determining which object is occluded and which object is occluding, thereby separating them into fore- and background, particularly in cases where the occluded object is larger than the occluding one. Auditory objects, in contrast, cannot truly occlude each other the way visual objects can in optics. In acoustics, sound energy from different sources adds linearly and creates a complex sound that has to be decomposed by the brain. This differs from the occlusion in vision, because the physical reflections of light that reach the retina are those of the

object that occludes and the exposed parts of the object that is being occluded. Most importantly, the part of the "background" object that is occluded is not transmitted to the retina. However, for sounds that merge, this analogy does not hold, because even in situations where one sound may mask another, the sound energy from the masked sound, despite being undetected behaviorally, still contributes to the sound wave that reaches the basilar membrane. Another major difference between visual and auditory scene segmentation is that, in vision, the object we focus (our attention) on occupies a restricted part of our retina. In audition, the sound we focus (our attention) on is still part of a merged sound wave and this complex sound will cover a range of the frequencies across the basilar membrane inside the cochlea. It is true that this range of activation inside the cochlea could be occurring in "patches", for example when I'm walking up St. Paul Street and the reversing truck's beeps occur at much higher frequencies than most of the background sounds at that point in time. However, when I am at a café and intending to have a conversation with my friend while there are another 30 or so active speakers, music and noise bursts from clattering dishes, most of the energy in the sound wave that reaches my ear will be in the frequency range of speech production and analysis. In other words, the sensory representations of auditory objects are less immediately-informative compared to that of visual objects: the retinal stimulation pattern already carries some local information about a visual stimulus, but basilar membrane activation does not. Instead, auditory information requires central processing of the time-frequency structure of sound patterns. A fitting analogy which Josh McDermott described previously (2009) is to imagine a world in which visual objects are not opaque, but see-through. Now

segmentation of different visual objects becomes considerably harder, because cues such as occlusion become unreliable and masking of objects turns into a real problem.

*Factors influencing auditory scene analysis*

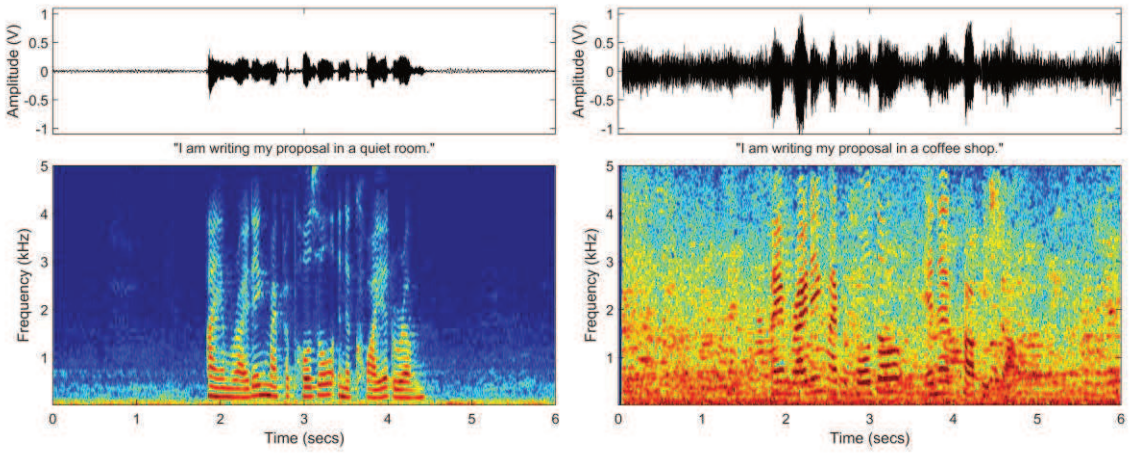
In a natural environment, sounds from multiple different sources mix, occur simultaneously and change rapidly. Despite the complexity of the resulting sound wave, normal-hearing listeners are typically able to follow a conversation in a crowded room. As discussed above, the segregation and formation of auditory streams (e.g. "voice of interest" versus "background noise") occurs based on simple acoustic features such as frequency changes across time, but is also influenced by other processes, like attention. It is important to note that sound source segregation and attention do not necessarily operate in a particular order, but that they are influenced by one another: what sound sources we segregate is partial to what we attend to (Bregman, 1990, Carlyon 2004). For example, if you are listening for a specific person's voice in a crowded room to localize them, you are using your prior knowledge of that person's voice (male/female, high-pitched/low-pitched voice, etc.) to steer your attention towards sounds that match this template. Yet, how effective you will be at suppressing the interfering voices is dependent on how well you can perceptually segregate different auditory stimuli (Carlyon, 2004). In other words, if the sounds in the scene are too similar to be perceptually distinguished, or if a listener does not know what features to listen for, "auditory search" becomes analogous to the pitfalls of serial search in vision: it is time-intensive and less robust with each additional signal added. At this point it seems appropriate to note that the major drawback of having to perform "auditory search" is the time component. In visual search, finding Waldo among hundreds of other cartoon

humans may take time, but the image itself does not change in the course of that time. In audition, listening for Waldo's voice among the chatter of everyone else means missing parts of the things Waldo says. As the listener samples more and more auditory streams in the hope to find Waldo's voice, additional auditory information is lost, which only stresses that the number of interfering sound sources creating the complex scene affect the listener's speed to switch their attention between sources, and thereby communicate effectively.

In situations where there is a single speaker in a quiet environment, or the sound of interest occupies a completely different frequency space than the otherwise present sounds, identifying the sound of interest is comparatively easy. Figure 2 shows the difference between a spoken sentence in two different environments. On the left, you see a visualization of me saying, "I am writing my dissertation in a quiet room," both as a waveform (top) and in spectrogram depiction (bottom). Here, you can clearly make out the fundamental frequencies and harmonics created by my uninterrupted and clear speech, and formants that accompany my articulatory features. By contrast, on the right side you see a visualization of the me saying almost the same sentence ("I am writing my proposal in a coffee shop"), but this time in a noisy environment that includes sounds produced by other people talking, glasses and mugs clinking, coffee machines hissing, etc. The amplitude in the left and right spectrogram depictions are normalized, so it becomes immediately evident – in both the waveform and the spectrogram views – that the previously easily detectable speech is now embedded in a cacophony of other sound signals that interact with the "target" signal (i.e. my speech). An increase in amplitude of the voices of nearby speakers, possibly even female speakers, or decrease in the intensity

of my voice would naturally further decrease the intelligibility of the spoken sentence – a situation that is commonly encountered at restaurants, parties or other social events.

**Figure 1. 2. Illustration of a spoken sentence in two different environments.**




Left: The sentence "I am writing my proposal in a quiet room" is uttered in an otherwise silent room and here represented as a waveform (top) and spectrogram (bottom). Fundamental frequencies and harmonic bands are clearly visible. Right: A similar sentence ("I am writing my proposal in a coffee shop") is uttered in a vibrant coffee shop and again represented as a waveform (top) and spectrogram (bottom). Fundamental frequencies are embedded in background noise.

This problem of speech intelligibility was first formally described as the cocktail-party-problem by Cherry in the 1950s (Cherry, 1953), and has been rigorously studied since then (e.g. Bregman 1990, Carlyon 2004, McDermott 2009). Noisy environments present challenges to sound segregation because the number of sound sources is large and their interaction creates a complex soundscape. McDermott describes the segregation of sound sources as an "ill-posed problem," because many solutions exist to reconstruct a complex waveform that has been created from multiple, linearly added separate sound sources (McDermott, 2009). Additionally, attentional selection of the correct sound source is complicated, as multiple items –not all of which are necessarily acoustic stimuli – are now competing for the listener's attention.

It is known that we employ physical components of sound to help separate different sound sources. For example, when a target sound comes from a different spatial location than the interfering sounds, it is easier for a listener to detect and follow the target signal (Cherry, 1953). In addition, visual cues can help disambiguate unclear speech: in noisy environments, we often look at a person's lips to understand their speech better. Past research has also shown that we solve challenging parts of the cocktail-party-problem by making use of statistical regularities and Gestalt principles (e.g. Bregman, 1990). For example, common changes in amplitude, especially across different frequencies, as well as onsets and offsets of signals tend to be reliable cues for grouping signals as belonging to the same sound (Best et al., 2007). In fact, the latter are part of the simultaneous grouping process described above. Figure 2 on the left shows such effects throughout the visualization of my speech: the harmonics that build upon the fundamental frequencies share amplitude changes, and on- and offsets. This helps to group each syllable or phoneme, or generally sounds, together. On the right-hand side of Figure 2 you can see that the fundamental frequencies are buried in background noise (0 ~ 500 Hz), but that the harmonic frequency bands and amplitude changes, as well as common on- and offsets throughout my speech are retained (~700 – 4000 Hz). Note that a few "breaks" between my words or phonemes may be filled with sound energy at similar frequencies from other sound sources, distorting the cues that are useful for separating sound sources and auditory stream formation. Here, Bregman's sequential grouping notion becomes important, as the coherent changes across time reveal which frequency components should be grouped together, versus ignored. Thus, we can ensure that successive sounds from the same single sound source are grouped together to form a

coherent auditory stream. Beyond such fundamental cues that influence grouping of sounds into streams, higher order perceptual components such as pitch and timbre and semantic structure help identify ambiguous signals (Bregman, 1990; Culling and Darwin, 1993). Further, in extremely noisy situations, visual cues, like lip movements as mentioned above, may help fill in gaps or clarify masked parts of a speech signal to create a complete understanding of the signal's original meaning (McDermott, 2009).

Overall, which auditory object captures the focus of our attention is inherently a combination of bottom-up saliency factors, such as the onsets, frequency changes, loudness or relevance of a sound, and top-down knowledge about the environment, such as wanting to listen to the speaker on one's opposite, or a female voice (Serences et al., 2005; Shinn-Cunningham, 2008). In fact, knowing what to attend to aids in enhancing the neural representation of those selected objects (Elhilali et al., 2007; Fritz et al., 2007).

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I will now introduce the echolocating bat as the subject of all experiments presented in this thesis. I will start out reviewing the diversity of bats, their echolocation signals, and how they adapt their sonar emission to different behavioral tasks. Subsequently, I will explain how bats use their biosonar vocalizations to localize objects in 3D space, and elaborate on the challenges of scene analysis.

*Echolocating bats*

The roughly 1,300 species of bats comprise about a fifth of all mammals (Fenton and Simmons, 2015; Wilson and Reeder, 2005). Contrary to a common misconception, about 160 species of bats use vision to navigate their environments, just as most mammals, including humans, do (Kunz and Fenton, 2005). However, the majority of bats have developed a specialized hearing system that allows them to probe their environment with sound (Griffin, 1958), instead of using vision.

*Echolocation & diversity of biosonar signals*

To navigate the environment, any organism needs to process sensory input from their surroundings. In some animals, this input is received passively via environmental stimuli, meaning that there is no active part in the sensing of the environment. For example, we open our eyes and light from some source reflects off objects around us, allowing us to detect their locations, shapes, colors, etc. In contrast, some organisms orient using active sensing mechanisms. For example, the electric fish emits brief electric pulses under water and senses changes in the electric field caused by objects in their surrounding (von der Emde, 1999), a process called electrolocation. Similarly, echolocation describes the process by which an animal produces a loud sound that propagates through a medium, and subsequently reflects off surrounding objects, returning sound waves with changed frequencies or time components. In addition to bats, echolocating animals include toothed whales (Au, 1993), birds (Brinkløv et al., 2013), and some humans (Stoffregen and Pittenger, 1995).

Different species of bats are found all over the world, except in polar regions, and have a diverse diet ranging from fruit, nectar and pollen, to insects, fish, frogs, smaller animals, other bats, and blood (Neuweiler, 1990; Simmons and Stein, 1980a). The ecological niches of bats can vary from open spaces without vegetation to densely cluttered forests, and echolocation signals are diverse across different species (Schnitzler and Kalko, 2001). The echolocation signals used by laryngeal echolocating bats can broadly be classified as *frequency-modulated (FM)* or *constant-frequency (CF)* signals, and can be emitted through either the nose or mouth. Different combinations of these two signals are found in natural bat broadcasts (e.g. CF-FM, FM-CF-FM). In general, FM signals are short (0.5 to 10 ms), downward-sweeping sounds with a broad bandwidth of about 30 to 80 kHz, and often have more than one harmonic (FM1, FM2, FM3). CF signals, on the other hand, are comparatively long (6-100 ms), narrowband signals that span about 1-3 kHz, and have a high duty-cycle (Simmons and Stein, 1980; Moss and Schnitzler, 1995; Schnitzler and Kalko, 2001). CF bats compensate for Doppler shifts introduced by their own flight speed, and further listen to Doppler shifts in returning echoes that are produced by fluttering insect prey (e.g. von der Emde and Menne, 1989; von der Emde and Schnitzler, 1986, 1990, Hiryu et al., 2005, 2007, 2008; Mantani et al., 2012; Schnitzler, 1973; Schnitzler and Flieger, 1983; Trappe and Schnitzler, 1982), whereas FM bats use time markers across frequencies to analyze the spectral patterns of returning echoes (e.g. Bates et al., 2011; Simmons, 1973).

*Biosonar signal adaptation*

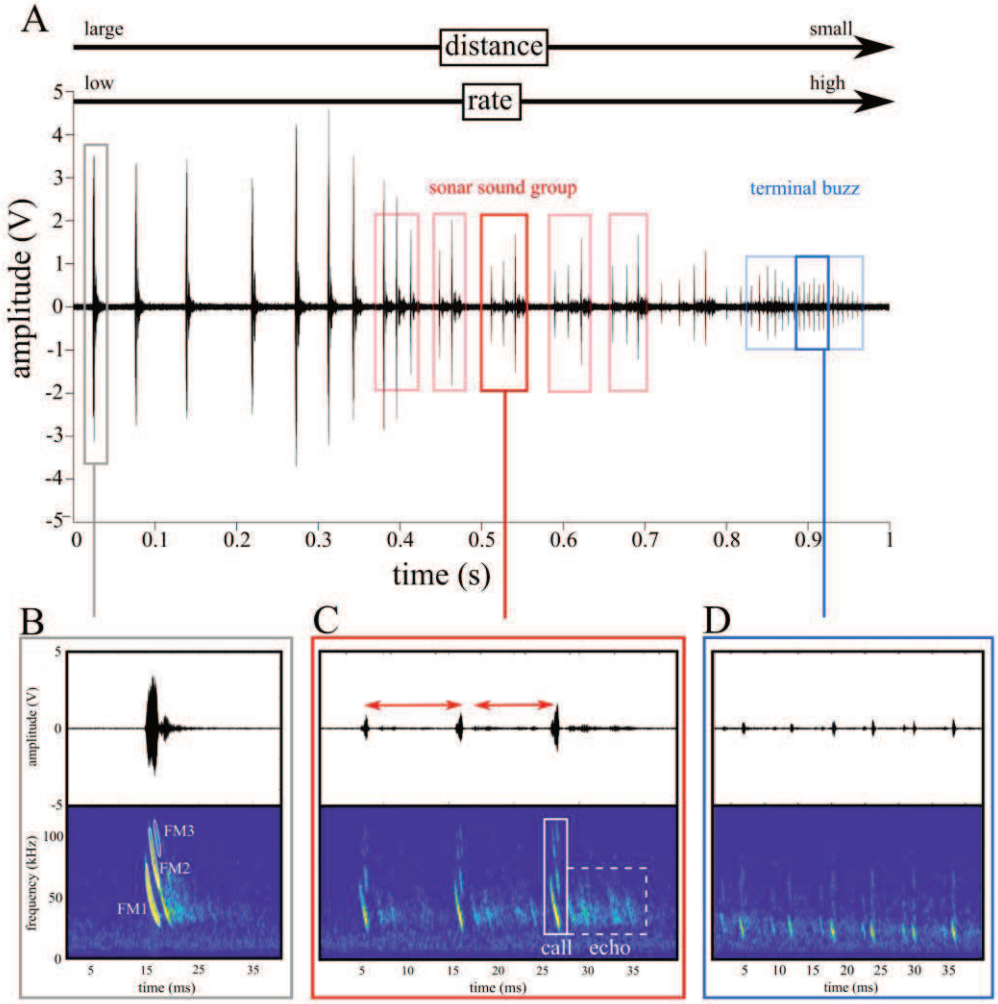
While echolocating bats navigate through their environment, they dynamically adapt the duration, amplitude and frequencies of their calls, as well as the timing between calls

(Figure 3). These adaptations are in response to closing in on a target object: bats reduce the duration of their calls as they close in objects to avoid call-echo overlap, a situation in which an object may be so close to the bat that it could return an echo while the bat is still emitting a sound (Kalko and Schnitzler, 1989); they reduce the amplitude of their calls to keep the prey's echo strength the same (Hartley, 1992); they increase the bandwidth of their calls to better localize the target object and perceive its shape, size and texture (Hartley, 1992; Simmons et al., 1995b; Simmons, 1973), and lastly, they decrease the timing between sonar emission (and thereby increase the call rate) to receive more frequent information of the target object's location and movements (Griffin, 1958; Surlykke and Moss, 2000).

All bat sonar signal adaptations are part of a dynamic process, changing with the current task at hand and distance to the target object. For example, when FM bats set out to search for prey in open spaces, they emit shallow, narrowband and comparatively long calls at low call rates of about 5-10 Hz (Fig. 3 (A) grey box, (B); Griffin, 1958; Moss and Surlykke, 2001; Simmons et al., 2001; Surlykke and Moss, 2000). Upon detection of insect prey, bats decrease the call duration, increase the call bandwidth and rate, oftentimes creating what is commonly referred to as "sonar sound groups": clusters of a few calls at a stable and consistent pulse interval (PI), which are flanked by longer intervals (Fig. 3 (A) red boxes, (C); Kothari et al., 2014; Moss et al., 2006). Such sound groups are commonly observed when bats abruptly change their flight direction (Moss et al., 2006), prepare for capture and may need high spatial resolution (Kothari et al., 2014), or fly among high acoustic clutter – objects in the bat's environment (Moss et al., 2006; Petrites et al., 2009; Warnecke et al., 2016). When bats are about to capture prey or

prepare for landing, they emit calls at steady, short 5 – 8 ms intervals, a rate of about 150 – 200 Hz, which is commonly referred to as the "buzz phase" (Fig. 3 (A) blue boxes, (D); Moss et al., 2006; Simmons et al., 2001).

**Figure 1. 3. Illustration of an FM bat's echolocation sequence as it captures prey.**




(A) As distance from the bat to the target object decreases ("distance" arrow), call rate increases ("rate" arrow). A typical sequence of calls (black) as recorded of a bat capturing a tethered mealworm in a flight room is plotted across time (x-axis). Boxed are different portions of the bat's typical sequence: search (0 to 0.3 seconds), with long duration calls (gray) and intervals, approach (0.3 to 0.8 seconds), with shorter duration calls and intervals and sonar sound groups (red), and capture (0.8 to 1 second), with short calls at steady intervals, called buzz phase (blue). (B) Oscillogram and spectral representation of a call (gray box) with three harmonics indicated (FM1, FM2, FM3); from grey box in (A). Calls in search phases are typically long and shallow in the field. Recordings in the lab can differ from this pattern, as the environment is more restricted. (C) Oscillogram and spectral representation of call (white box) and echoes (white dashed box) within red box in (A). Calls are part of a triplet sound group. (C) Oscillogram and spectral representation of calls within blue box in (A). Calls show a narrow bandwidth at low frequencies and occur at a stable ~5 ms interval.

The emitted sounds of the bat are directional (e.g. Hartley and Suthers, 1989; Jakobsen et al., 2013), which restricts the perception of 3D space to areas ensonified by the sonar beam. Overall, the big brown bat's sonar beam extends to about 70 degrees for frequencies in FM1 (22-50 kHz), and 40 degrees for frequencies in FM2 (44-100 kHz) at -6 dB down from the peak (Hartley and Suthers, 1989). In order to spatially sample their environment sufficiently, big brown bats must control the aim of their sonar beam. Previous work demonstrated that the bat narrows the angles of its beam aim as it approaches a target object, and locks its beam onto the target in the last 0.5 to 1 m before target capture (Falk et al., 2011; Ghose and Moss, 2003). More recent work showed that the bat controls the width of its sonar beam by lowering the emitted frequencies and thereby increasing the ensonified area of space during insect capture (Jakobsen and Surlykke, 2010).

*Sonar localization*

Most mammals, including humans, use interaural time and level differences (ITD and ILD, respectively) to determine the position of a sound source in azimuth (Schnupp et al., 2011). The echolocating bat actively ensonifes its surroundings and *creates* the sound sources it perceives. Early research has established that the big brown bat, *Eptesicus fuscus*, can discriminate a 1.5° of angular separation between two thin rods in azimuth, which corresponds to about 0.3 to 1.5 dB of ILD, and 1 μsec of ITD (Moss and Schnitzler 1995). The maximum ITD of the same species has been estimated to be about 40-50 μsec, the maximum ILD about 25-30 dB (Moss and Schnitzler 1995).

Vertical sound localization cues, on the other hand, depend on the outer ear reflections introduced to the spectrum of the sound (Simmons et al. 1995, Schnupp et al.

2011). This notion underlines species-specific differences, as some bats have large pinnae that can be moved independently to increase level differences between their two ears (Moss and Schnitzler 1995). Previous work has confirmed the pinna and tragus's role in vertical sound localization and revealed a discrimination ability of 3° angular separation between objects at different elevations (Chiu and Moss, 2007; Griffin, 1958; Lawrence and Simmons, 1982; Wotton et al., 1995).

In a third spatial dimension, bats accurately and continuously determine their dynamically changing distance to the target object. Simmons (1973) was the first to demonstrate that the bat uses the time delay between an outgoing call and a returning echo to estimate the distance to the target object. Simmons (1971, 1973) showed that the big brown bat can discriminate ranges of about 12 mm (~ 70 μsec) at different absolute distances.

In natural environments, spreading loss and atmospheric attenuation contribute to the degradation of the emitted echolocation signal in the field such that small objects at great distances would be hard to detect (Stilz and Schnitzler, 2012), and even harder to classify. This suggests that FM bats may designate small fluttering insects at large distances as "prey" rather than identify a specific representation of the object (Moss and Schnitzler, 1995). By contrast, CF bat echolocation is exceptional at the detection of weak fluttering echoes due to strong spectral modulations (Kober and Schnitzler, 1990), even if the insect is embedded within high acoustic clutter.

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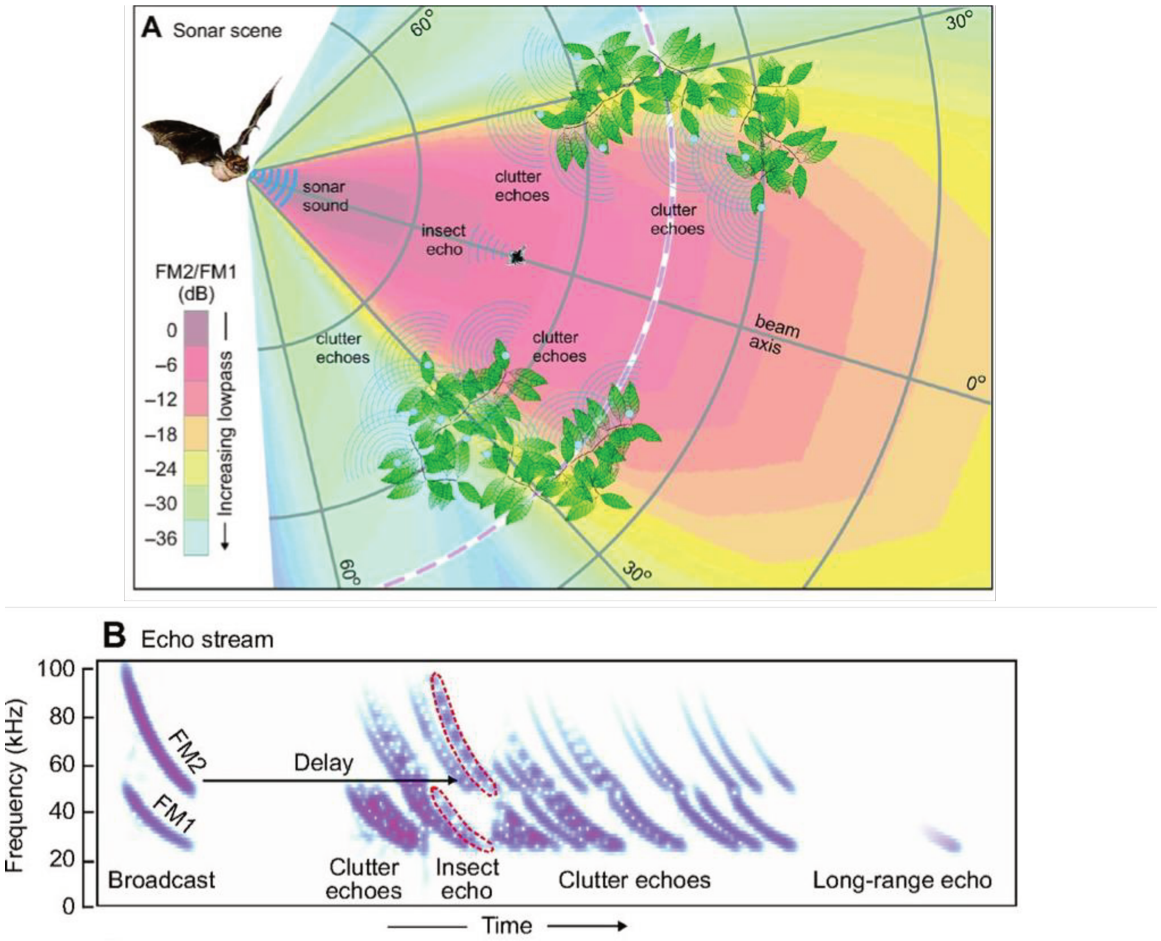
In the following section, I elaborate on the general challenges that bats face when analyzing their acoustic scene. In this capacity, I will introduce two mechanisms that bats employ when foraging among other bats. Subsequently, I will introduce the notion of echo flow, which describes dynamic changes of an acoustic scene that may influence bat flight and echolocation behavior.

*Acoustic scene analysis in the echolocating bat*

The ability of an echolocating bat to successfully forage in its natural environment is highly dependent upon the signal-to-noise ratio of the echo signal it receives. There are several sources of signal degradation. For example, the originally emitted call is attenuated (with the magnitude dependent on distance) before interacting with an object, returning a weak echo signal, the echo may be embedded in noise that is created by other nearby bat calls and echoes from their signals, or it may interact with echoes from other background structures that surround the target prey's immediate location (e.g. shrubs, leaves). As such, it is evident that noise and background echoes influence the bat's perception of its auditory scene. To successfully forage in its environment, the bat needs to sort echoes from different objects and correctly determine which part of the complex sound wave is the representation of its target object (See Figure 4).

**Figure 1. 4. Illustration of a bat's physical and acoustical soundscape.**




(A) Bat emits a biosonar call that has different beam widths, depending on the frequency (see scale). Objects in the bat's environment (leaves, branches insect) return echoes to the bat at different time points. The echo of the target prey (insect) may return to the bat after echoes of other objects at closer distances to the bat return echoes. (B) Spectrogram of the scene depicted in (A). The bat's broadcast is indicated as having two strong harmonics (FM1, FM2). After some delay, echoes from objects at different distances return to the bat, some overlapping in time creating spectral notches (white dots inside harmonics). In the cascades of echoes that are depicted, the bat needs to detect and identify the echo that belongs to the insect (outlined in red). Figure is used with permission from James A. Simmons.

Previous work has investigated the extent of acoustic interference in the bat's immediate environment, which influences the bat's ability to detect and sort sonar signals, and strategies used to minimize acoustic detection by a conspecific competitor. First, bats have been shown to dynamically adapt the spectro-temporal characteristics of vocalizations in situations of multiple bats foraging together (e.g. Obrist, 1995; Chiu et

al., 2009). Second, bats sometimes exhibit silent behavior, the ceasing of biosonar call emissions, which been reported as a possible mechanism to increase chances of foraging success in competitive settings (Chiu et al., 2008).

*Changes of spectro-temporal features*

It has been reported that bats modify the spectral-temporal features of their vocalizations in response to the presence of conspecifics (Chiu et al., 2009; Obrist, 1995; Ulanovsky et al., 2004). In field studies, recordings were collected while bats flew in their natural environment, and showed that when flying in groups, bats produce calls that had different frequency or temporal patterns than when flying by themselves (Obrist, 1995; Ulanovsky et al., 2004). In a more controlled laboratory study, Chiu et al. (2009) recorded flight and echolocation calls of single and paired bats flying in an open laboratory environment. Chiu and colleagues report systematic changes of frequency and duration of biosonar vocalizations that are dependent on the call similarity of bats as recorded in single flight trials (Chiu et al., 2009). In other words, the more similar two bats' call characteristics are (as measured by start- and end frequency, bandwidth, duration and sweep rate), the more these bats change such characteristics when paired and left to compete for a single prey item. Additionally, a playback study in *Tadarida brasiliensis* showed that this bat raises the end frequency of their FM sweep in response to playback jamming signals (Gillam and McCracken, 2007). Independently, it has been shown that *Eptesicus fuscus* changes the end frequency of FM calls when calls are emitted in a densely-cluttered environment, so as to distinguish echoes from subsequent calls more effectively (Hiryu et al., 2010), which adds clarity of echo detection to the list of possible reasons for end frequency adaptations.

*Silent behavior*

Changing spectro-temporal features of biosonar emissions appears to be a reliable mechanism to aid sorting calls and echoes between foraging conspecifics. Additionally, silent behavior has been reported as another mechanism that may be of importance during prey capture tasks in paired bats (Chiu et al., 2008). In this study, when two bats competed for a single prey item in an open laboratory space, one would cease to echolocate ~ 40% of the time. This "silent behavior" was defined as not emitting any vocalizations for at least 200 ms. At an average flight speed of about 3 – 5 m/s, this means that a given bat did not emit sounds for *at least* 0.6 – 1 m of its flight path; in one case Chiu and colleagues report silence for 70% of a trial, about 2 full seconds. While this silent behavior surely decreases the complexity of the acoustic scene that any bat has to analyze, it can be argued that it may not be used in order to facilitate prey detection and minimize interference, but to "eavesdrop" on the continuously echolocating bat searching for the prey, while not giving away its own position: most commonly, silent behavior was observed when two bats were in a following-flight configuration and it was consistently the following bat that ceased to echolocate. Commonly also, it was the bat that was last silent which caught the prey, suggesting that silent behavior may be a form of "stealth foraging behavior" to increase chances of prey capture in competitive situations. Currently, the true function of silent behavior is still unknown.

*Echo flow*

In a manner similar to visually-guided animals' use of optic flow cues to steer locomotion, acoustically-guided animals may use acoustic, or echo flow cues. For

example, as an echolocating bat navigates through its environment, it emits intense, high frequency sounds and listens to echoes that return from objects within its surroundings. A bat that navigates through a densely cluttered forest, will receive echoes from trees and bushes at different distances. Depending on the density of these trees, the return of these echoes may be rather short in time delay (for dense spacing of trees) or long (for sparse spacing of trees). At any point in time, a single biosonar emission will thereby result in a cascade of echoes returning to the bat, and depending on the density of these echoes, the bat may change its flight or echolocation pattern.

To date, only a few studies have investigated the effect of cascades of echoes on navigation (e.g. Petrites et al., 2009; Warnecke et al., 2016; Wheeler et al., 2016; Aharon et al., 2017), and it is yet unclear what the exact nature of flight adaptations is. Petrites and colleagues created a densely-cluttered environment by hanging several chains from a ceiling and leaving a small corridor for the bat to navigate through (Petrites et al., 2009). Manipulating the number of chains that cluttered the bat's immediate environment, they showed that the big brown bat decreases its call rate and flight speed with increasing clutter density, and also increases the number of sonar sound groups in high clutter conditions. Wheeler et al., (2016) used a similar setup but manipulated the width of the corridor that the bat could fly through from 1 m to ~ 40 cm (about 5 cm larger than this bat's wing span), and reported shorter pulse intervals and more sonar sound groups when bats flew through narrower corridors.

In a recent study, Aharon et al., (2017) tested bats flying in long (~ 40 m) corridors that was lined by plastic poles, to investigate the question whether *Pipistrellus kuhlii* uses acoustic flow information to estimate distance. They found that acoustic flow

in their experiment was not a major component of this bat's estimation for distance, which indicates that it may serve a different purpose.

While the information gathered by these behavioral experiments help our understanding of how general echo cascades influence the bat's echolocation behavior, none of these studies systematically varied the number of echo cascades returning to the bat and measured their flight behavior. Experiments that more carefully manipulate these cascades of echoes can expand our understanding of sonar guidance and be useful in creating autonomous navigation systems. Studies investigating the behavioral adaptations to different controlled patterns of echo flow are outlined in Chapters two, three and four below.

*The Current Study: Goals*

For this thesis, I utilized two approaches to investigate how changes in the acoustic scene might influence bat navigation and echolocation behaviors.

First, in Chapters 2, 3 and 4 I manipulated a controlled echo-acoustic environment to understand how these changes influenced behavior. Specifically, in **Chapter 2**, I exposed the bat to a controlled corridor environment in which its flight path was restricted, and the echo-acoustic information could be manipulated systematically. Inspired by research investigating changes in flight to different optic flow cues, I created a corridor from individually moveable poles and varied the spacing between poles on either side of the corridor to be either balanced or imbalanced. This setup returned

different patterns of echo-acoustic flow to the bat as it flew down the corridor and received cascades of echoes specific to the pole manipulation. I measured how the flight and echolocation behaviors adapted as a function of manipulation of pole spacing.

Subsequently, in **Chapter 3**, I extended the results on behavioral adaptations to echo-acoustic flow from the study in Chapter 2 to investigate the underlying mechanisms that may have been driving these flight adaptations. Specifically, I used felt to attenuate echo cascades returning to the bat, to test whether the intensity of the echo stream was a major influence in the bat's behavioral adaptations. Additionally, I recorded neural patterns in the bat inferior colliculus (IC) of awake-restrained big brown bats while playing sound recordings from the echo flow corridor, to test whether the timing between echoes within cascades influenced the bat's processing of cascades.

While these studies inform us about the effects of different echo flow patterns on flight behavior in the big brown bat, we do not know much about how behavior would be impacted in species that use a different kind of echolocation signal. The big brown bat emits short frequency-modulated signals, but other species, such as *Hipposiderid armiger terasensis,* use long constant-frequency biosonar calls. This bat forages in dense vegetation as well as along forest edges, suggesting that it may be specifically-adapted for navigation of highly-cluttered environments, like the echo flow corridor. To learn more about how differences in signal design might influence behavior in the same environment, in **Chapter 4** I present behavioral data of the Taiwanese leaf-nosed bat, *Hipposiderid armiger terasensis*, navigating through the same echo flow environment discussed in Chapters 2 and 3.

In my second approach to investigate behavioral adaptations to changes in the acoustic scene, I let pairs of bats fly in different environments, and measured the changes in their echolocation parameters as they competed for single a prey item. Specifically, I focused my analyses on “silent behavior”, an approach taken by bats during foraging situations, which was first formally described by Chiu et al. (2008). In **Chapter 5**, I present an extension of previous work by Chiu et al. (2008, 2009), and aimed to understand how the prevalence of silent behavior changes when bats fly in cluttered environments. Surprising results of that study motivated the work presented in **Chapter 6**, in which I extended the work presented in Chapter 5 by additionally varying food availability and controlling the gender of bats.

Finally, in n **Chapter 7**, I discuss the results of all studies carried out for this thesis, and focus in on a few key points that are important to consider for future work.

# Chapter 2

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# The effect of echo flow on bat flight and echolocation behavior

Completed Experiment Synopsis: All animals use sensory feedback to plan and guide their subsequent movements. Previous work in the big brown bat has shown that it adapts its flight and echolocation behavior in response to the type of environment it navigates. However, no study has systematically explored how the density of echoes effects the bat's behavior. Here, we studied how the flight and echolocation behavior of the big brown bat is affected when navigating through a corridor of individually movable poles. Manipulation of the spacing between poles, or the number of poles, allowed us to test the effect of echo density and echo cascades on bat behavior. We show that the echolocating big brown bat deviates towards the corridor side with fewer echoes returning to it.

**Title: Dynamic echo information guides flight in the big brown bat**

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*Abstract*

Animals rely on sensory feedback from their environment to guide locomotion. For instance, visually guided animals use patterns of optic flow to control their velocity and to estimate their distance to objects (e.g. Srinivasan et al. 1991, 1996). In this study, we investigated how acoustic information guides locomotion of animals that use hearing as a primary sensory modality to orient and navigate in the dark, where visual information is unavailable. We studied flight and echolocation behaviors of big brown bats as they flew under infrared illumination through a corridor with walls constructed from a series of individual vertical wooden poles. The spacing between poles on opposite walls of the corridor was experimentally manipulated to create dense/sparse and balanced/imbalanced spatial structure. The bats' flight trajectories and echolocation signals were recorded with high-speed infrared motion-capture cameras and ultrasound microphones, respectively. As bats flew through the corridor, successive biosonar emissions returned cascades of echoes from the walls of the corridor. The bats flew through the center of the corridor when the pole spacing on opposite walls was balanced and closer to the side with wider pole spacing when opposite walls had an imbalanced density. Moreover, bats produced shorter duration echolocation calls when they flew through corridors with smaller spacing between poles, suggesting that clutter density influences features of the bat's sonar signals. Flight speed and echolocation call rate did not, however, vary with dense and sparse spacing between the poles forming the corridor walls. Overall, these data demonstrate that bats adapt their flight and echolocation behavior dynamically when flying through acoustically complex environments.

*Introduction*

To navigate in the natural environment, an animal must estimate its relative distance to obstacles along its path in order to avoid collision and reach its goal. Previous research in flying animals, such as honeybees (Baird et al., 2005; Srinivasan et al., 1996, 1991) , budgerigars (Bhagavatula et al., 2011) and *Drosophila* (Tammero and Dickinson, 2002) has provided evidence that these visually-guided species rely on optic flow, the angular velocity of image motion across the animal's eyes, to guide locomotion (Srinivasan et al., 1991). Optic flow provides continuous feedback to an animal about its relative velocity and distance to objects in its environment, and experimental manipulations of optic flow cues produce changes in animal flight trajectory and speed (Baird et al., 2005, 2010; Bhagavatula et al., 2011; Dyhr and Higgins, 2010; Linander et al., 2015a, 2015b; Scholtyssek et al., 2014; Srinivasan et al., 1996, 1991).

In the dark, optic flow cues become unreliable, and flying animals may need to rely on other strategies or sensory cues to move through a complex environment. For example, some bats have evolved an active sensing system – echolocation – to exploit echo-acoustic information to guide movements in the dark. Echolocating bats produce acoustic signals in the ultrasonic range and extract features of the environment from information carried by echo returns from surrounding objects. Some bat species use constant frequency (CF) signals, which are well suited to measure relative velocity (Müller et al., 1999; Schnitzler, 1973), while other species rely exclusively on frequency modulated (FM) signals, which are well suited for spatial localization (Moss and Schnitzler, 1995; Simmons et al., 1995a; Wotton et al., 1995). Bats compute the distance

to objects from the time delay between sonar emissions and echo returns (Simmons, 1973) and the angular offset of objects from inter-aural difference cues (Lawrence and Simmons, 1982; Moss and Schnitzler, 1995; Simmons et al., 1983). Populations of neurons in the bat auditory system show selective responses to 3D spatial acoustic information, i.e. distance (pulse-echo delay), azimuth and elevation, providing the neuro-computational substrate for dynamic sonar scene representation (reviewed in Dear et al., 1993; Suga, 1990; Ulanovsky and Moss, 2008). A recent neurophysiological study in the FM bat, *Phyllostomus discolor*, reports changes in echo-delay tuned neural responses to playbacks of pulses and echoes meant to simulate patterns of echo flow that a bat might receive as it flies past an obstacle (Bartenstein et al., 2014). At the behavioral level, changes in echo delay, inter-aural differences and Doppler cues must be integrated across time from successive vocalizations to render updated echo scenes. The intermittent sampling of spatial information through echolocation occurs at intervals spanning tens to hundreds of milliseconds and contrasts with the nearly continuous sampling of information through vision.

The big brown bat, *Eptesicus fuscus*, is a nocturnal mammal that flies at speeds between 2 to 6 m/s (Falk et al., 2014), while probing its environment with short, downward-sweeping frequency modulated (FM) biosonar sounds, which contain several harmonics in the range of 25 to 130 kHz (FM1: ~65 to 25 kHz, FM2: ~130 to 50 kHz) and last between 0.5 to 15 ms (Fenton and Bell, 1981; Griffin, 1958; Surlykke and Moss, 2000). This bat dynamically decreases the duration and interval between sonar emissions as it approaches and intercepts prey (Simmons et al., 1979), and must rapidly process changing echo information to guide appropriate adjustments in flight and echolocation

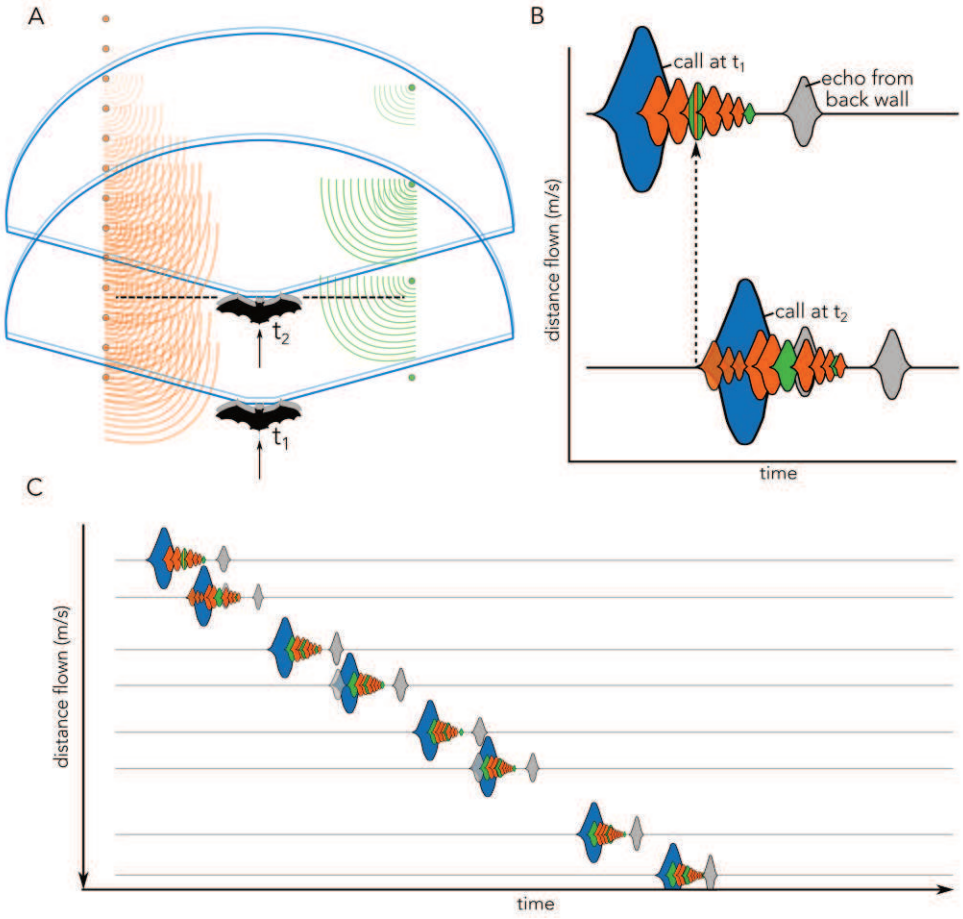
behavior (Surlykke and Moss, 2000; Simmons et al., 2001, Moss and Surlykke, 2010). Previous studies have shown that bats adapt echolocation call features in response to information extracted from echoes, which in turn influences the subsequent biosonar call design. For example, bats that produce CF signals adjust the frequency of their echolocation calls to compensate for Doppler shifts introduced by their own flight velocity (Schnitzler, 1968, 1973; Neuweiler et al., 1980). Both CF and FM insectivorous bats adapt the emission rate, duration, and bandwidth of their sonar signals, depending on changing distance to prey in both open and cluttered environments (Schnitzler et al., 2001; Simmons et al., 2001, 1979; Surlykke et al., 2009a; Surlykke and Moss, 2000). While performing obstacle avoidance and prey capture tasks that demand high spatial localization accuracy, FM bats adjust their call rate and produce sonar sound groups, clusters of echolocation calls with short intervals, flanked by calls at longer pulse intervals (Moss et al., 2006; Petrites et al., 2009; Kothari and Wohlgemuth et al., 2014; Sändig et al., 2014). Therefore, echoes from objects in the bat's environment directly affect the bat's call timing, which in turn, directly influences the temporal patterning of echo returns. Laboratory research further demonstrates dynamic adjustments in flight speed, which are coordinated with adaptive echolocation behaviors (Petrites et al., 2009; Falk et al., 2014). For example, Petrites et al. (2009) show that the big brown bat decreases its call rate and flight speed with increasing clutter density, and they report an increase of sonar sound groups in high clutter conditions. Falk et al. (2014) further report shorter call durations in cluttered compared to open flight spaces. Overall, these results suggest that the big brown bat dynamically adapts its flight speed and biosonar call parameters when flying in cluttered environments.

Adjustments in bat flight and echolocation behaviors can be related to the echo-acoustic scenes the animal perceives via echolocation. Following each vocalization, a bat receives a cascade of echoes from objects ensonified by its sonar beam (Fig. 1(**A**)). The horizontal beam of the big brown bat is spatially broad and spans approximately ± 40-70 degrees, depending on sound frequency (-6 dB beam width, Hartley and Suthers, 1989). Therefore, when the bat moves through its environment, the broad sonar beam will often ensonify not only prey, but also other objects. The bat's analysis of its echo scene thus involves the integration and segregation of cascades of echoes arriving from different objects at different spatial locations and distances (Moss and Surlykke, 2010). This is a particularly challenging task, as echoes from closely spaced objects may overlap in time, creating complex interference patterns (Simmons et al., 1990; Bates et al., 2011).

Past studies of echolocation behavior in complex environments challenged the bat to maneuver around obstacles, which introduces uncontrolled echo-acoustic variables that are difficult to quantify across individual animals (e.g. Falk et al., 2014). In this study, we attempt to address this limitation by investigating the bat's echolocation and flight behavior in experimentally controlled corridors, which constrain the animal's flight trajectory and allow echo-acoustic information to be systematically varied. As the bat flies in the controlled corridor, it experiences differential changes of returning echo patterns over a series of echolocation calls. These echo cascades arrive at the ears of the moving bat and vary with the animal's velocity, its head aim, and distance to objects in the environment (Fig. 1). We here refer to these changes in echo patterns as "echo flow". While the construction of our bat flight corridor was inspired by animal studies of

visually-guided flight, it is important to note that fundamental differences between vision and echolocation preclude direct comparisons between optic flow and echo flow.

**Figure 2. 1. Cartoon illustrating the concept of echo flow as used in the present article.**




(A) Bat (black, $t_1$) enters the tunnel and emits a vocalization (blue). Echoes return to the bat from the left (orange) and right (green) corridor sides. In the time that the bat emits the call (blue) it moves (grey), thereby slightly displacing the vocalization (light blue) and creating complex echo patterns. By the time the bat sends out its second call (black dashed line, $t_2$), it has travelled further down the corridor, but some echoes from the previous call ($t_1$) may still be returning to the bat. These echoes will overlap with the subsequent call emission (blue, $t_2$) and echoes returning from call 2. (B) Biosonar vocalizations illustrated as sounds waveforms (blue) and echoes returning to bat from poles located at different distances (orange) from both corridor walls (orange vs. green) are shown across time (x-axis). Grey echo illustrates the echo from the wall at the end of the tunnel (see Fig. 2(A)). The onset of call 2 (call at $t_2$) occurs when echoes from call 1 (call at $t_1$) are still arriving (dashed black arrow). The distance that the bat has flown in between two calls in schematically plotted on the (y-axis). Calls (blue) and echoes (orange/green/grey) are matched between panels A and B. Note that head movements are not displayed and may further complicate echo patterns. (C) Schematic of waveforms across time (x-axis) as the bat flies down the corridor and emits successive vocalizations (blue). Pulse-echo overlap may occur (e.g. call 2, 4,6), complicating the spectral and temporal echo structures. The wall echo (grey) will increase in intensity and move closer to the time of vocalization as distance to the end of the corridor decreases. Y-axis plot distance flown between successive calls. Note that waveforms in (B) and (C) are cartoons illustrating the complex merging of the echoes returning to the bat; they do not represent how the bat perceives the flow of echoes.

In the present study, we investigated the effect of echo flow patterns on bat flight and biosonar behaviors. Echolocating big brown bats flew through a corridor with walls constructed from individual poles, mimicking rows of trees that a bat might encounter in its natural environment. This setup allowed us to manipulate the echo patterns of each corridor wall that each bat would receive as it flew. We hypothesized that the density of poles comprising each corridor wall would influence bat flight trajectory and echolocation behavior. Specifically, we predicted that bats would fly along the midline of the flight corridor with balanced pole-spacing on opposite walls, and that they would show wall-following behavior in conditions with imbalanced pole-spacing. We further hypothesized that the bats would adapt their call duration and use of sonar sound groups with the density of poles comprising the corridor walls. Specifically, we predicted that bats would shorten call duration and increase sound group production when they flew through corridors with walls comprised of more densely spaced poles.

## *Material and Methods*

### *Animals*

Seven wild-caught big brown bats, *E. fuscus* (three males and four females) were individually trained in an empty flight room to fly through a hole cut into a custom-built foam wall (Figure 2**(A)**, inset; see below). Bats were fed with mealworms (*Tenebrio molitor*) daily to maintain their individual weights between 13 to 16 g for the period of training and testing. The animals were housed in two group cages under reversed 12-hour light/dark-cycle in a colony room kept at 24 to 28 degrees C at 40 to 50 % relative humidity. The experimental procedures were approved by the Johns Hopkins University

Institutional Animal Care and Use Committee.

*Experimental Paradigm*

Experiments were carried out in a custom-built carpeted flight room (6 x 7 x 2.5 m), with the walls and ceiling lined with acoustic foam (Sonex Classic, Sonex Acoustics, San Jose, USA) and shielded from outside electrical noise. Along the diagonal of the flight room a corridor was built from individually moveable wooden poles, 2.5 cm in diameter each that spanned from floor to ceiling (Figure 2(A), grey circles). The corridor measured 6.2 m in length and 1.2 m in width. Each pole was individually set up and straightened by referencing to a Bosch self-leveling Line Laser (Robert Bosch Tool Corporation LLC., Michigan, USA). The left and right walls of the corridor were constructed with experimentally controlled spacing of the poles (Fig. 2). Four conditions with different configurations of pole spacing on the left and right sides of the corridor were tested in the experiment (Fig. 2(B)). At the opening to the corridor, a black felt curtain with an elliptic hole (31 x 38 cm) (Rainbow Felt Black, Fabric.com, USA), attached to a large frame (251 x 120 cm) of acoustic foam prevented the bat from gaining information about the wall configurations until the animal began its flight through the corridor on each trial (see Fig. 2(A), black line labeled “felt curtain”, and grey bar and inset labeled “frontal view”).

Prior to each experiment, the designated bat was removed from its cage and allowed to fly for several minutes in an adjacent flight chamber. Once the bat was actively flying, water-soluble glue (Grimas Mastix Water Soluble, Heemstede, Holland)

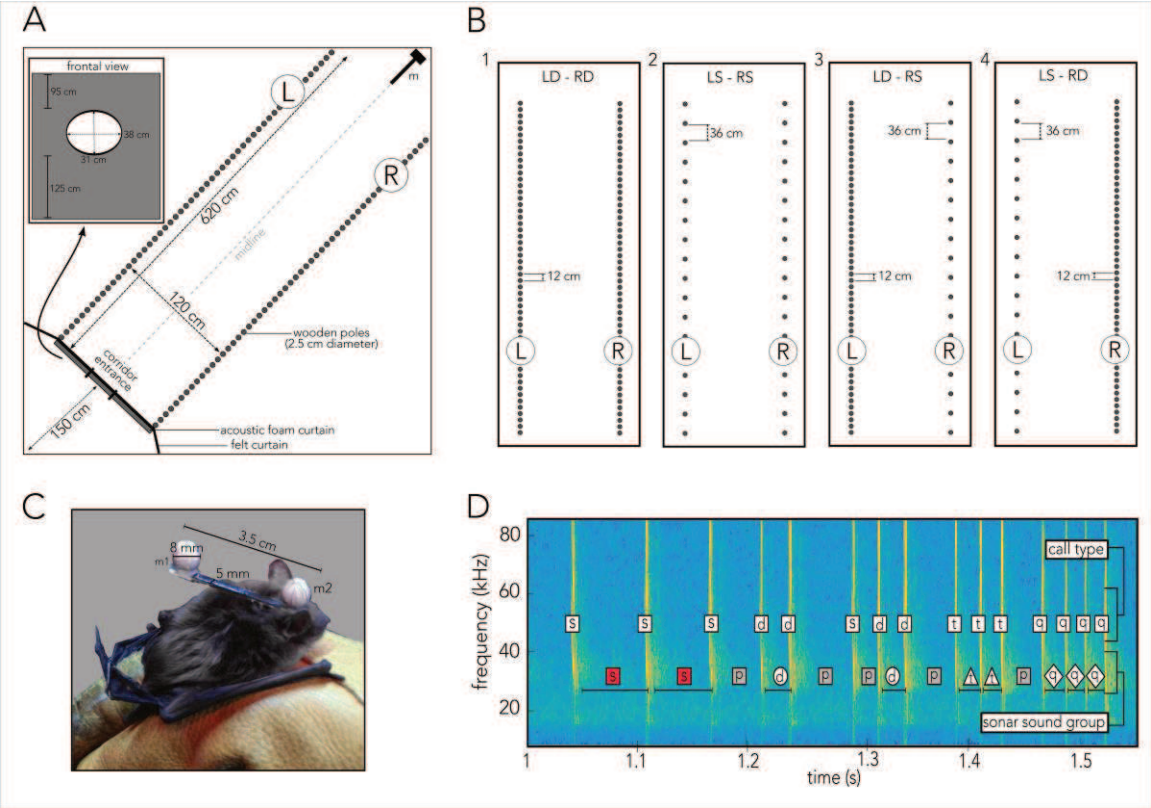
was used to attach a custom-built head marker to the bat's head (Figure 2**(C)**). The head marker was 3.5 cm in length and 5 mm in width. Two reflective spheres (diameter: 8 mm) were glued to each end of the marker, so that one sphere was positioned between the bat's ears (Fig. 2(C), m2), and the other one was positioned on the bat's back (Fig. 2(C), m1). The total weight of the marker was 0.9 g, which corresponds to about 5-7 % of body weight of individual bats. When the glue had dried and all recording systems had been configured, the experiment started. After collecting data over at least ten trials, the head and body markers were carefully removed, and the animal was returned to its cage. Every day the seven different bats were tested in the same order and at the same time. For each trial three experimenters were present: the first experimenter released the bat from behind the curtain and otherwise stayed in that location. A second experimenter was responsible for catching the bat after a trial had ended and safely return it to the curtain-enclosed space. The "catcher" was otherwise waiting between the corner of the felt curtain and the flight room wall (Fig.2). The third experimenter recorded notes on every trial and triggered recording system for trial capture (see below).

Each bat navigated four different corridor configurations (Fig. 2(B)) over a minimum of 10 trials. To study the bat's flight and echolocation behavior in response to the density of clutter and the flow of echo information, we tested each animal in combinations of dense and sparse spacing of wooden poles. Due to the duration it took to prepare each corridor setup, a given condition was tested on a single day. For all conditions, dense spacing refers to a 12 cm gap between two poles, and sparse spacing refers to a 36 cm gap between two poles (see Fig. 2(B)). Bats were released at ca. 1.5 to 1

m distance from the acoustic foam curtain, and they entered the corridor by flying through the entrance hole. Each of the seven bats navigated through a corridor whose two walls were comprised of densely-spaced (Fig. 2(B), 1) or sparsely-spaced (Fig. 2(B), 2) poles. These corridor wall configurations served as baseline conditions. To test the effect of imbalanced left/right echo patterns on behavior, each animal also flew through a corridor in which one wall was comprised of sparsely-spaced poles (Fig. 2(B), 3R; 4L) and the other one of densely-spaced poles (Fig. 2(B), 3L; 4R).

To restrict bats from using visual cues (Hope and Bhatnagar, 1979), all data collection was done in a dark room that was solely illuminated with dim infrared light for motion-tracking detection of the two reflective markers on the bat. Measurements of the light levels in the flight room at the beginning, middle and end of the corridor each revealed a light intensity of $<10^{-2}$ lux. Measurements were done using a spectrophotometer (GS-1500, Gamma Scientific, San Diego, USA) at experimental conditions.

**Figure 2. 2. Experimental procedures.**




(A) Cartoon of the 6 x 8 x 2.5 m flight room, covered in acoustic foam, housing a corridor (620 cm x 120 cm) created from individual wooden poles (grey circles) so that the corridor's left (L) and right (R) walls could be independently manipulated. Bats had been trained to fly through an elliptic hole (inset labeled "frontal view") embedded in a rectangle of acoustic foam and black felt to prevent them from gaining echo information about the pole spacing prior to entering the corridor. The felt extended to the walls of the flight room (black line labeled felt curtain). 13 high-speed IR motion-tracking cameras were mounted within the tunnel (omitted for visual clarity) to capture the bat's flight path, pole and microphone positions. Four ultrasonic microphones (m) were mounted vertically along the height of the room at the end of the tunnel. (B) Each of the seven bats flew at least ten trials in each of the following four different tunnel configurations. For each configuration "dense" refers to a 12 cm spacing of wooden poles, "sparse" refers to a 36 cm spacing of poles. 1) Left dense, right dense (LD-RD), 2) Left sparse, right sparse (LS-RS), 3) Left dense, right sparse (LD-RS), 4) Left sparse, right dense (LS-RD). (C) Photograph of the actual head marker, measuring 3.5 cm in length and 0.5 cm in width (original background greyed out) on the bat. Each reflective ball (m 1, m 2) to be tracked by a motion-tracking system is 8 mm in diameter. Head marker weighed 0.9 g total, making up about 5-6% of the animal's body weight. Marker was attached using water-soluble theater glue. (D) Spectrogram illustrating sonar sound groups and call types as defined in the present study. Time series of calls is plotted as frequency (kHz, y-axis) across time (seconds, x-axis). For each call, the call type is indicated in the upper white box, while each sonar sound group type is indicated in the lower box. For sound groups, box shape and color correspond to marker shape and color in Figure S2. Codes: s = single call/ PI, d = doublet call/PI, t = triplet call/PI, q = quadruplet call/PI, p = pre-/post-sound group PI.

*Data Recording*

For each trial, synchronized audio and motion-tracking data of the flying bat were captured. Audio data were recorded using 4 ultrasonic microphones (D500X external microphone, Pettersson Elektronik Uppsala, Sweden) bandpassed between 10 and 100 kHz, mounted at the end of the corridor (Fig. 2, m). Audio data were sampled at 250 kHz (NI PXI board 6143). The bat's flight trajectory was acquired through 13 high-speed IR motion-capture cameras (Nexus, Vicon, Vicon Motion Systems Ltd., UK) mounted on the ceiling within the corridor. The motion-tracking system tracked the two reflective spheres attached to each bat at 300 frames per second. After all trials for the day were collected, the motion-tracking program also collected data on the position of the microphones, the location of the entrance hole, and the poles that made up the corridor walls. Every trial was manually triggered by an investigator after the bat had traversed the corridor at full length. Data acquired within 6 seconds prior to the trigger were stored for analysis.

Data from the motion-tracking system were processed offline, and custom-written MATLAB programs (Mathworks, Natick, MA, USA) were used to digitally analyze the audio data and 3D flight tracks of the bat.

*Data Processing and Analysis*

Motion-capture data were processed with custom-written MATLAB code to reconstruct 3D tracks of each bat's navigational patterns on a given day. In subsequent processing, we used the 2D projection of the bat's flight path onto the horizontal plane (floor) to compute its deviation from the midline of the corridor (Fig. 2**(A)**, grey dashed

line). Positive numbers indicate a deviation to the right side of the corridor, negative numbers indicate a deviation to the left side (see Results). Data points are calculated as distance from the end of the corridor, which has been defined as the plane created by the last poles on the left and right sides (Fig. 2(A), grey circles closest to 'm').

Echolocation calls produced by bats flying in the corridor were manually processed using an open-source MATLAB package that is archived on the GitHub software repository at https://github.com/leewujung/call_marking_gui. We extracted the start and end times of each call that was emitted during the portion of the flight path that had been previously reconstructed. With these parameters we then calculated the call rate, pulse interval, and duration of each call.

For all variables of interest, data points at distances smaller than 1 m from the end of the corridor and greater than 6 m from the end of the corridor (the "start" of the corridor) were excluded. Only data collected from the middle portion (a total of 5 m) of the corridor were analyzed.

We used JMP to perform analyses with a mixed effects model on the relationship between experimental condition and parameters of interest (deviation from the midline, flight speed, call rate, call duration, pulse interval). A Tukey's range test (HSD) was used for post-hoc testing. For analysis of flight speed and sound parameters, we collapsed the two conditions that create an imbalanced flow of echoes (LS-RD, LD-RS) into one condition (S/D), as these conditions are essentially identical with regard to those variables. Unless otherwise noted, the mixed effects model analysis used condition (N = 4 for track data, N = 3 for all other data) as fixed effects and bat (N = 7) as random effects. Z-tests to test the deviation from the midline (zero-point) were done across

condition and trials. A total of 312 trials (LD-RD: N = 67, LD-RS: N = 64, LS-RD: N = 68, LS-RS: N = 62, LDS-RD: N = 51 trials) were analyzed.

*Results*

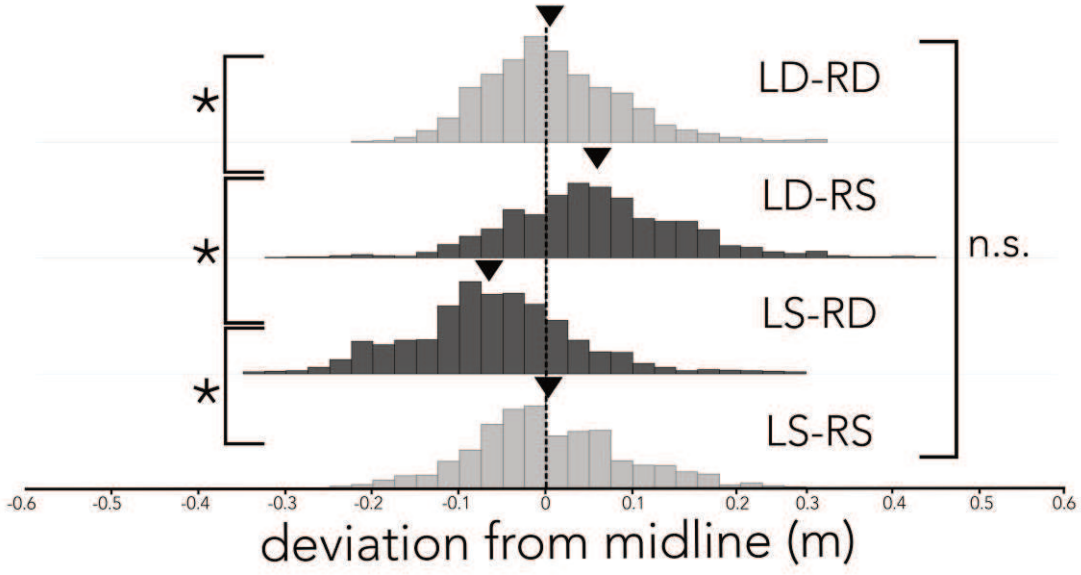
Here we report how the spacing of poles along the corridor walls influenced the bat's flight and echolocation behaviors. All flight data are plotted according to the bat's deviation from the midline. Thus, negative numbers represent deviations to the left of the midline, whereas positive numbers represent deviations to right of the midline.

*Flight tracks*

Figure 3 shows the bats' average deviation from the midline (dashed black line) in the corridor across different conditions. When the corridor consisted of densely spaced poles on both sides (Fig. 3, LD-RD), the bats centered their flight path and on average (black triangle) deviated 0.0075 m from the midline toward the right side. Similarly, when the corridor was comprised of sparsely spaced poles on both sides (Fig. 3, LS-RS), the bats stayed close to the midline, deviating on average (black triangle) 0.0013 m from the midline. In both conditions, where the spacing of poles on either side of the corridor was imbalanced (Fig. 3, LS-RD, LD-RS) the bats steered away from the wall with more densely spaced poles. Statistical analyses confirm a difference in the distributions of deviation from the midline across conditions. Using a Tukey's HSD test we report that flight paths in LS-RD and LD-RS differ from baseline conditions (LD-RD, LS-RS) and from one another ($F_{3,18} = 21.597$, $p < 0.0001$; LD-RD: M = 0.0075 m, SE = 0.0004, LS-

RS: M = 0.0013 m, SE = 0.0006, LD-RS: M = 0.055 m, SE = 0.0006, LS-RD: M = -0.0607, SE = 0.0005). The baseline conditions did not differ from one another. Importantly, flight paths in LS-RD and LD-RS also differ from zero (i.e. the midline of the corridor; z-test, LS-RD: z = -2.25, $p = 0.012$; LD-RS: z = 1.94, $p = 0.026$). In contrast, neither LD-RD nor LS-RS differ from zero (i.e. the midline of the corridor; z-test, LD-RD: z = 0.226, $p = 0.82$, LS-RS: z = -0.26, $p = 0.79$).

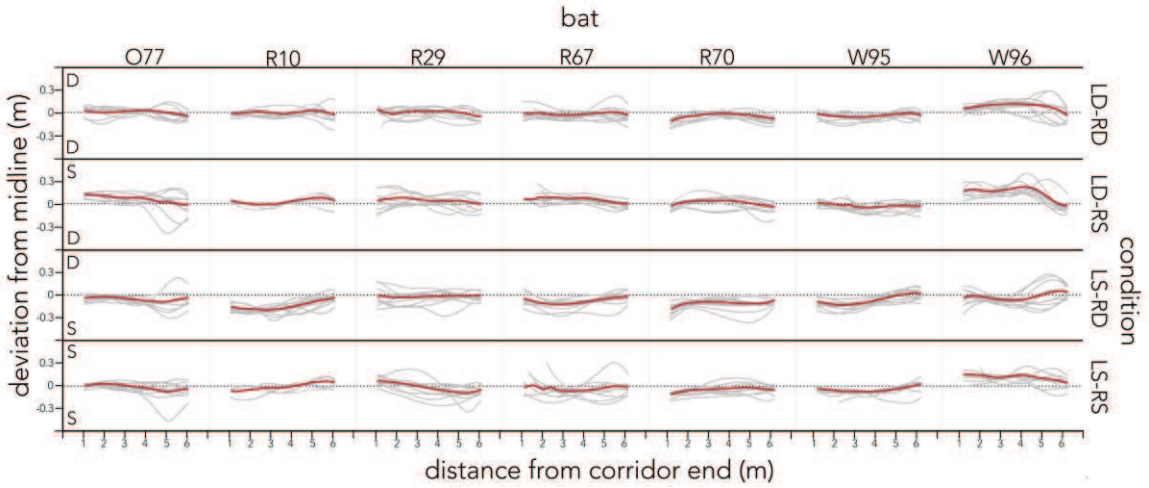
**Figure 2. 3. Distribution of flight path deviation from the midline across conditions.**




Histogram of the distribution of deviation (x-axis) from the midline (black dashed line) across all conditions (LD-RD, LD-RS, LS-RD, LS-RS). Data are plotted in 2.5 cm bins, and balanced pole-spacing conditions (LD-RD, LS-RS) are illustrated in light grey, imbalanced pole–spacing conditions (LD-RS, LS-RD) are illustrated in dark grey. Average deviation from midline is indicated by a black triangle. Bats' flight path differs significantly between acoustically unbalanced conditions (LS-DR, LD-SR), and unbalanced conditions differ significantly from balanced conditions. Balanced conditions (LD-RD, LS-RS) do not differ significantly.

Figure 4 plots the raw flight tracks (grey) and their mean (red) of each bat along the entire corridor for each condition.

**Figure 2. 4. Distribution of raw flight tracks across conditions and bats.**




For each condition (right y-axis) raw flight tracks (grey) and their mean (red) are plotted as distance from the corridor end (lower x-axis). Tracks are plotted as deviation from the midline (black dotted line) along the left y-axis. The letter in the corner of each condition indicates the spacing of poles on that side of the condition. All data are plotted for each of the seven bats (upper x-axis). Bats' flight paths steer away from densely-spaced corridor walls and center otherwise.

*Flight speed*

Figure 5(A) compares the bats' flight speed (circles and solid line) and their call rate (squares and dashed line, see below) across flight corridor configurations. On average, bats navigated all conditions at around 3.8 m/s. Flight speed did not differ across conditions ($F_{2,12}$ = 1.77, $p$ = *0.21*; LD-RD: M = 3.76 m/s, SE = 0.0054, LS-RS: M = 3.74 m/s, SE = 0.006, S/D: M = 3.83 m/s, SE = 0.004).

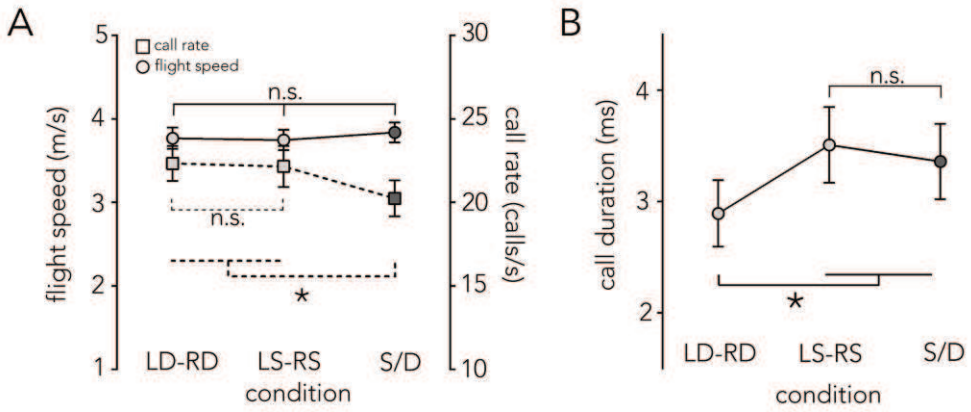
*Echolocation sampling*

Previous research has shown that a change of flight speed in the echolocating bat is often accompanied by a reciprocal change in call rate (Petrites et al., 2009; Falk et al., 2014). We did not find a change in flight speed and also found that the call rates remained constant across baseline conditions, but showed a slight decrease in acoustically

imbalanced conditions (S/D) ($F_{2,12}$ = 5.7531, $p$ = *0.017*; LD-RD: M = 22.33 calls/s, SE = 0.077, LS-RS: M = 22.15 calls/s, SE = 0.096, S/D: M = 20.67 m/s, SE = 0.061, Fig. 5(A)).

Overall, calls are shorter in the LD-RD condition (M = 2.89 ms, SE = 0.32) compared to the LS-RS condition (M = 3.5 ms, SE = 0.32) and S/D (3.36 ms, SE = 0.32) ($F_{2,12}$ = 26.35, $p < 0.001$, Fig. 5(B)).

**Figure 2. 5. Flight speed, call rate and call duration across conditions.**

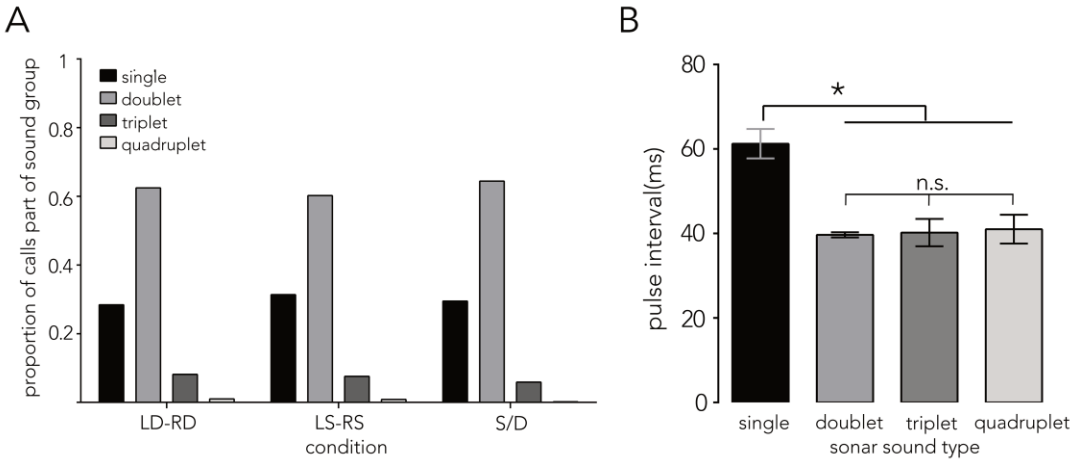



(A) Mean flight speed ±1 SE in m/s (left y-axis), is plotted across conditions (x-axis). When the animals fly though the different corridor manipulations (LD-RD, LS-RS, S/D) flight speed (circles, solid line) is relatively stable at around 3.8 m/s with no significant differences across conditions. Also plotted is the mean call rate per second ±1 SE (right y-axis) across conditions (squares, dashed line). There are no significant differences in call rate in baseline conditions, though the difference between S/D and both baseline conditions is significant. (B) Mean call duration ±1 SE (y-axis) is plotted for each condition (x-axis). Bats use significantly shorter calls in the LD-RD condition compared to all other conditions (LS-RS, S/D).

Of the 5,854 calls analyzed across conditions, 70.3% were part of a sound group (see Figure 6(A), doublet, triplet and quadruplet), with calls creating a doublet call group making up the largest proportion (~ 62%). The distribution of pulse intervals (PIs) was split into doublet sound groups (a single interval between 2 calls, Fig. 2(D), "d"), triplet sound groups (two intervals between three calls, Fig. 2(D), "t"), quadruplet sound groups (three intervals between four calls, Fig. 2(D), "q") and single sound groups (intervals

between single calls that did *not* immediately precede or follow another sound group (Fig. 2(D), red "s", Figure S2, red). Single pulse intervals that immediately preceded or followed a sound group (~76%, Fig. 2(D), "p", Fig. S2, black) were excluded from this analysis. The overall sound group distribution was analyzed using a random effects model with condition (N = 3) and sound group (N = 4) added as fixed effects, and bat (N = 7) added as random effects. Figure 6(B) illustrates the mean pulse intervals used within each type of sound group. We did not find an effect of condition ($F_{2,58}$ = 0.2831, $p$ = *0.75*, LD-RD: M = 46.48 ms, SE = 3.78, LS-RS: M = 44.80 ms, SE = 3.69, S/D: M = 47.65 ms, SE = 3.78), but report that pulse intervals differ significantly across sound group types: pulse intervals between single sounds (red "s" in Fig. 2(D)) are longer (M = 61.25 ms, SE = 3.74) compared to intervals between sounds that make up a sound group ($F_{3,59.49}$ = 12.60, *p<0.0001*, doublet: M = 39.68 ms, SE = 3.74, triplet: M = 40.20 ms, SE = 3.74, quadruplet: M = 44.10 ms, SE = 5.86).

**Figure 2. 6. Sonar sound groups and pulse interval distributions.**




(A) Proportion of calls that are either single calls (single) or part of a sonar sound group (doublet, triplet, and quadruplet) is plotted on the y-axis across conditions (x-axis). Around 62% of all calls are doublets. (B) The mean pulse intervals ±1 SE (PI, y-axis) that bats use when navigating the different corridor configurations show differences across sound group types (x-axis). Pulse intervals between single sounds (see red "s" in Fig. 2(D)) have a significantly larger PIs of around 60 ms. By contrast, doublets, triplets and quadruplets share a shorter PI around 40 ms. Data are pooled across conditions, as no difference across conditions (LD-RD, LS-RS, S/D) was found.

*Discussion*

Animals exploit a rich array of sensory cues to find their way in the environment, relying on vision, hearing, olfaction, somatosensation, infrared and magnetic sensing (Schone, 2014). Early work has suggested that animals relying primarily on vision for navigation, guide their movements through optic flow in order to measure their locomotion with respect to the objects in their surroundings (Gibson, 1958; Koenderink, 1986). Studies investigating the effect of optic flow on animal flight have provided evidence that several species (bees, budgerigars, and fish, among others) adapt their movement trajectories and speed in response to experimentally controlled optic flow cues (Srinivasan et al., 1991; Srinivasan et al., 1996; Baird et al., 2005; Baird et al., 2010; Bhagavatula et al., 2011; Scholtyssek et al., 2014; Linander et al., 2016).

Here we investigated how an acoustically-guided animal, the echolocating big brown bat, adapts its flight and sonar behavior in a restricted environment that returns dynamically changing patterns of echo flow. Inspired by paradigms used in optic flow research, we asked how the bat would change its flight trajectory, speed and biosonar behavior in a corridor with walls of differing in echoic structure. In this corridor, each sonar emission returns a cascade of echoes from objects at different distances, some overlapping in time, which creates complex echo flow patterns (see Fig. 1). These patterns change dynamically as the bat emits successive sounds in flight. It is noteworthy that acoustic flow in the corridor contrasts with optic flow along several dimensions. First, optic flow depends on relative movement between the animal and its environment,

and provides a cue for relative distance. Echolocating bats, on the other hand, compute distance to objects directly from echo delay (Simmons, 1973). Second, a visual animal's movement through the environment induces optic flow from light patterns present in the environment. In the case of echo flow, the dynamic features of echo cascades depend on the bat's active and discrete sampling of sensory information from the environment. Third, optic flow is independent of the contrast or intensity of visual patterns (Srinivasan et al., 1991) and unaffected by different spatial periods between 0.15 and 0.4 cycles $cm^{-1}$ (Dhyr and Higgins, 2010). By contrast, the structure and spatial configuration of corridor walls in our experiment directly influence the echo flow patterns the bat receives as it flies. Fourth, past research has shown that optic flow information can be experimentally manipulated or eliminated (e.g. Srinivasan et al., 1991; Scholtyssek et al., 2014). Echo flow information, however, cannot be removed from our flight corridor, whose walls return echoes that vary with the bat's distance and directional aim of the sonar beam. These differences therefore limit direct comparisons between animal studies of optic flow and echo flow.

In accordance with our experimental predictions, the bats flew closer to the side with wider pole spacing when the corridor walls had different inter-pole spacing on the left and right sides (Fig. 3, LS-RD and LD-RS). In conditions where the spacing was identical on both corridor walls, bats centered their flight paths, though they showed more variability in their flight trajectories along the corridor with wider inter-pole spacing (Fig. 3, LS-RS vs. LD-RD), suggesting that reduced acoustic reflections from the walls influenced flight path planning. Our results show that bats deviate from the midline, away from the wall with dense pole spacing and towards the wall with sparse pole

spacing. By contrast, bees continue to fly along the midline of a tunnel that displays different spatial frequencies of black and white vertical stripes on either side (Srinivasan et al., 1991). However, it is important to note that this independence of spatial frequency on the bee's centering behavior holds only for differences in spatial periods between 0.15 and 0.4 cycles $cm^{-1}$ (Dhyr and Higgins, 2010). For vertical grating patterns outside of this range, bumblebees deviate towards the wall displaying higher spatial frequency stripes, which contrasts the bat's behavior of veering away from the higher density wall configuration in our experiment. Note, however, that the changes of spatial frequency patterns utilized by Dhyr and Higgins (2010) should only be carefully compared to the changes of pole spacing in our study, as we manipulated the distance between poles, and not the width of poles on each side.

When bees and budgerigars are presented with corridor walls that display patterns of vertical stripes on one side and horizontal stripes on the other, animals adapt their flight paths to show wall-following behavior, as optic flow cues are absent from the wall displaying horizontal stripes (Srinivasan et al., 1991; Bhagavatula et al., 2011; Scholtyssek et al., 2014). As noted above, it is not possible to remove echo information from a structured environment, as all physical objects, including walls, return echoes, which are influenced by the bat's distance to those objects as well as the directional aim of their sonar beam. In a previous study, bats abolished centering behavior in a flight corridor with horizontal and vertical poles on opposite walls (Figure S1(A)), flying closer to the wall with horizontal poles (Fig. S1(B)) that return fewer echoes (Fig. S1(C)), LH-RV, LV-RH; Warnecke and Moss, 2015). While the overall bat flight paths in this earlier experiment mirror the flight adjustments observed in visually-guided animals flying

along corridors with vertical and horizontal stripes on opposite walls (e.g. Srinivasan et al., 1991; Bhagavatula et al., 2011; Scholtyssek et al., 2014), the findings of both research fields must be interpreted independently, as echo flow is not eliminated in horizontal pole condition.

Overall, bats produced shorter duration echolocation calls when flying through corridors with dense pole spacing (Fig. 5(B)), which indicates that clutter influences sonar behavior. Specifically, shorter duration calls in highly cluttered conditions suggest a strategy that the bat might employ to reduce pulse-echo overlap from multiple, closely-spaced reflecting surfaces (see Fig. 1(A, B); Petrites et al., 2009). Contrary to our experimental predictions, flight speed and echolocation call rate in baseline conditions did not vary with differences in the corridor wall pole spacing (Fig. 5(A)) though on average, bats emitted fewer calls in corridors comprised of imbalanced echoic walls. The observed constant velocity suggests that the bat can adapt its flight behavior (deviation from the midline to steer away from dense pole spacing) without altering its velocity; this is consistent with studies on zebra fish swimming through manipulations of optic flow corridors (Scholtyssek et al., 2014).

Contrary to our prediction, the bats' call rate did not increase from sparse (LS-RS) to dense (LD-RD) pole spacing conditions, and the use of sound groups (reciprocal of call rate), was consistently prevalent (Fig. 6(A), Fig. S2). In fact, bats emitted very similar proportions of sonar sound groups in all trials across conditions, producing doublets ~ 62% of the time while flying through the corridor (Fig. 6(A), S2). The interval between single calls was significantly longer than the pulse intervals between clusters of

sounds (Fig. 6(B) single vs. doublet, triplet, and quadruplet). Past research suggests that sonar call groups may help the bat sharpen its representation of a complex environment (Kothari and Wohlgemuth et al., 2014), and the consistent prevalence of sonar sound groups recorded from bats flying in the corridor (Fig. S2) suggests that this environment presents the bat with a challenging echo scene that requires frequent sonar probing. Figure S2 illustrates typical and individual pulse interval patterns that each bat used when probing the corridor environment across conditions. It is noteworthy, that despite using different temporal patterning, the bats' flight behavior is very similar across conditions (Fig. 4).

Bats can use information carried by echoes to navigate narrow spaces, forage in cluttered environments, avoid objects in their path, and forage on the wing (Griffin, 1958; Simmons and Kick, 1983; Schnitzler, 1973). Similarly, visually-guided animals use optic cues to maneuver in complex environments (e.g. Srinivasan et al., 1991;Baird and Dacke, 2012; Linander et al., 2016). The flight adaptations to echo flow patterns and optic flow cues suggest that common principles may underlie guidance of animal movement planning, even though the physics of light and sound render the stimuli distinctly different. While research has demonstrated that visual animals adapt their flight trajectories to balance optic flow, it is yet unclear whether bats adjust their flight paths to balance echo flow, or to simply steer away from the more echoic corridor wall. More detailed investigations into these phenomena may help elaborate on the computations involved in sensory-guided movement.

*Supplementary Material*

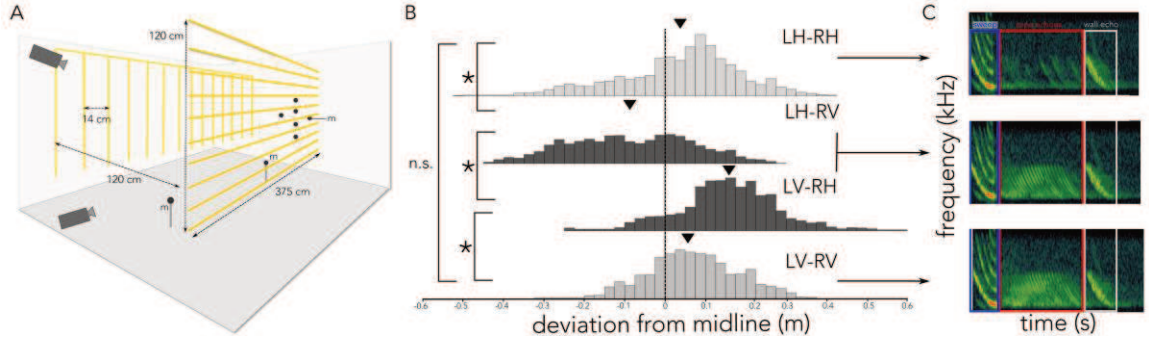
We provide supplementary data illustrating the results of an earlier experiment (Warnecke and Moss, 2015) in which bats flew through a 375 cm long x 120 cm wide corridor built from 1-inch diameter PVC pipes, arranged either vertically or horizontally, and hung from the ceiling using fishing line (Power Pro Spectra, Braided fishing line, Power Pro, Irvine, CA). Each corridor wall was 120 cm high and hung from the ceiling. Six ultrasonic microphones were mounted at the end of the corridor and two additional microphones were located within the corridor to capture the bat's echolocation behavior. Two high-speed IR cameras were mounted at the entrance of the corridor to record the 3D-flight path of each bat as it flew through the corridor. All trials were conducted under dim, long-wavelength ( > 650 nm) light to prevent the bats from using visual cues. In each trial, bats were released from the experimenter's hand at the entrance to the corridor, flew through the setup and the previous 4 seconds of synchronized audio and video data were recorded after triggering when the bat had exited the corridor.

Different corridor wall manipulations were tested. In two baseline conditions, bats flew through either 1) two vertically hanging PVC pipe walls (14 cm spacing, LV-RV) or 2) two horizontally hanging PVC pipe walls (14 cm spacing, LH-RH). In experimental conditions, bats flew through corridors with horizontal and vertical pipes comprising opposite walls (LH-RV, LV-RH).

Data processing and analysis was the same as in the experiment reported in this manuscript.

The results show that bats' flight behaviors are significantly different between the two baseline conditions (LH-RH: M = 0.04, SE = 0.017, LV-RV: M = 0.07, SE = 0.017) and the experimental conditions (LH-RV: M = -0.0862, LV-RH) ($F_{3,12}$ = 34.48, $p < 0.0001$). Neither baseline condition is different from zero (z-test, LH-RH: z = 0.659, $p = 0.25$, LV-RV: z = 1.446, $p = 0.92$). While, as expected, LH-RV differs from zero (z-test, z = 2.575, $p = 0.005$), the broad distribution of flight paths in LV-RH does not render it significantly different from zero (z-test, z = -1.272, $p = 0.10$).

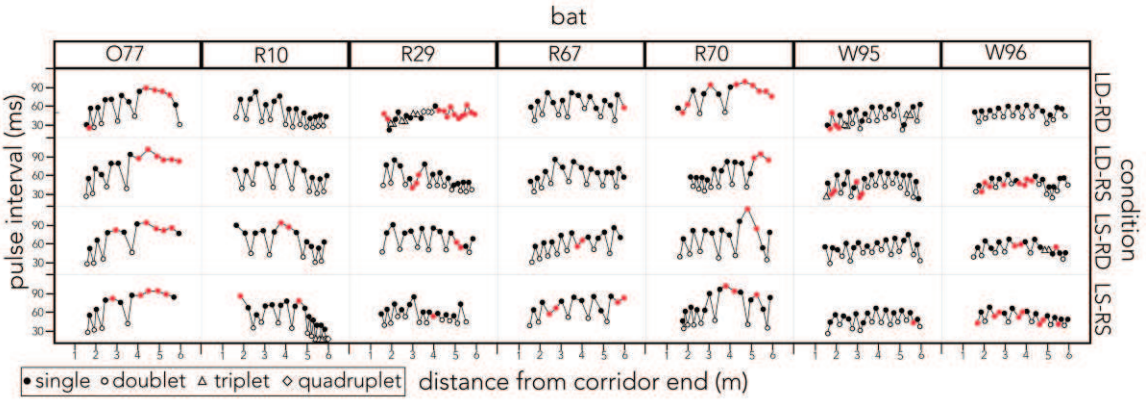
**Figure 2. 7. S1 Experimental paradigm and results of earlier echo flow experiment.**




(A) Schematic of the experimental setup illustrating a 375 cm long x 120 cm wide tunnel built from PVC pipes (yellow) that were combined into walls which could be individually hung from the ceiling. Walls were 120 cm in elevation. Individual poles were 14 cm apart in both vertical (here: left) and horizontal (here: right) walls. Bats' flight patterns were recorded with two high-speed cameras and echolocation behavior was captured using 8 ultrasonic microphones (m). Bats were released from experimenter's hand at the entrance to the corridor. Schematic depicts only one of 4 run conditions (LV-RH, see description above.) (B) Results of flight path deviations show that bats flew closer to the midline in baseline conditions (LH-RH, LV-RV), compared to experimental conditions (LV-RH, LH-RV). In experimental conditions bats deviated away from the vertically-spaced side and towards the horizontally-spaced side. **(C)** Spectrograms of echo measurements from the PVC corridor. Ultrasound signals were broadcast through an ultrasound loudspeaker (Ultra Sound Advice S56, London, UK) placed at the corridor entrance. The loudspeaker emitted a computer-generated hyperbolic frequency-modulated sweep, approximating the biological sonar signal of the big brown bat. Echoes were recorded with ultrasonic microphones (Ultra Sound Advice S56, London, UK) at the location of the loudspeaker. Spectrograms of echo returns in the LH-RH corridor (top) show fewer echoes (red) from the generated sweep (blue), compared to the imbalanced conditions (LH-RV, LV-RH, middle), and the LV-RV condition (bottom). Each spectrogram shows a pronounced wall echo (grey).

We further provide a data figure to illustrate the different temporal patterns of echolocation calls made by individual bats as they traversed the corridor in each condition. Plotted is one sample trial per bat, per condition.

**Figure 2. 8. S2 Individual temporal patterning of bat echolocation calls.**




Each bat (top x-axis) shows different, but consistent, temporal patterning of pulse intervals (PI, left y-axis) across conditions (right y-axis) and corridor length (x-axis). Filled circles indicate single pulse intervals (refer to Fig. 2(D)), red circles indicate single PI, black circles indicate pre-/post- sound group PI (Fig. 2(D), "p"). Pre-/post- pulse intervals are those that immediately precede or follow a sound group (either doublet, triplet or quadruplet, cf. manuscript). Open circles indicate doublet PI, triangles indicate triplet PI, diamonds indicate quadruplets.

# Chapter 3

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## What influences behavioral adaptations to echo flow?

Completed Experiment Synopsis: In a recent study, Warnecke et al. (2016; Chapter 2) investigated how acoustic information guides flight in the big brown bat. The animals flew through a corridor whose walls were built from vertically oriented poles, and spacing between poles could be manipulated. When the spacing of poles was symmetric on opposite walls, bats centered their flight paths in the corridor, but when the pole spacing was asymmetric on opposite walls, bats steered towards the wall with wider gaps between poles. While these results suggest that the flow of echoes returning from the corridor walls guides the bat's flight behavior, the intensity of echo returns could also be a contributing factor. Here, we tested this hypothesis by combining different pole spacings with manipulations of echo intensity by wrapping poles in sound-absorbing felt. The results show that intensity does not influence flight adaptations. We additionally recorded neurophysiological data in the big brown bat's inferior colliculus (IC) to recordings of the echo flow corridor to learn more about processing of echo flow. The results revealed that the timing of individual echoes in cascades shapes the representation of sonar soundscapes to guide navigation.

**Title: Echo interval and not echo intensity drives bat flight behavior in structured corridors**

Michaela Warnecke, Silvio Macias, Benjamin Falk and Cynthia F. Moss

Submitted to *Proceedings of the National Academy of Sciences*, April 2018

*Abstract*

To navigate in the natural environment, animals must adapt their locomotion in response to environmental stimuli. An acoustically-guided animal, the echolocating bat, relies on auditory processing of echo returns to represent its surroundings. Recent studies have shown that echo flow patterns influence bat navigation, but the acoustic basis for flight path selection remains unknown. To investigate this question, we released bats in a flight corridor with walls constructed of wooden poles. We manipulated pole spacing by changing the distance between poles (dense and sparse), and echo intensity, by wrapping felt around densely spaced poles. We predicted that bats would adapt their flight paths to deviate toward the side returning weaker echoes. Our results show that the bat's trajectory through the corridor was not affected by the felt manipulation, and bats deviated toward the corridor side with bare sparse poles. Walls with sparse pole spacing returned echo cascades at 2 ms echo intervals. By contrast, walls with dense pole spacing returned echo cascades with echo intervals less than 1 ms. To investigate auditory processing of echo cascades, we measured local evoked auditory responses in the bat inferior colliculus to echo playback recordings from the corridor. We predicted that neural responses would be reliably modulated by discrete echoes separated by at least 2 ms intervals within cascades, but not by echoes at shorter intervals within cascades. The data confirm this prediction and suggest that the bat's temporal resolution of echo cascades drives its flight behavior in the corridor.

*Introduction*

Navigation and orientation in the environment is fundamental to all organisms, and they must adapt locomotion in response to dynamic sensory stimuli. For example, optic flow, the angular velocity of image motion across the retina (Gibson, 1979), provides continuous feedback to an animal about its relative velocity and distance to objects in its environment (Srinivasan et al., 1996, 1991). In several behavioral studies, visually-guided animals, such as honeybees (Baird et al., 2005; Srinivasan et al., 1996, 1991), drosophila (David, 1982) and budgerigars (Bhagavatula et al., 2011), adapt their flight trajectory and speed in response to experimental manipulation of optic flow patterns (Baird et al., 2005, 2010; Bhagavatula et al., 2011; Dyhr and Higgins, 2010; Linander et al., 2015a; Scholtyssek et al., 2014; Srinivasan et al., 1996, 1991).

Animals that do not primarily rely on visual cues for navigation or object avoidance must use other sensory cues to move through a complex environment. The echolocating bat emits intense, high-frequency biosonar calls and exploits echo-acoustic information from objects in its environment to guide movements in the dark (Griffin, 1958; Neuweiler, 1990). Bats rely on binaural differences in arrival time and intensity to localize objects in the horizontal plane (Simmons et al., 1983); spectral cues to localize objects in the vertical plane (Lawrence and Simmons, 1982) and echo delay to estimate their distance to objects (Simmons, 1973).

Echoes from closely spaced objects in cluttered environments may overlap in time and create complex interference patterns (Bates et al., 2011; Simmons et al., 1990). The bat thus needs to successfully integrate and segregate cascades of echoes arriving from

objects at different spatial locations and distances (Moss and Surlykke, 2010). Previous research has shown that echolocating bats navigate densely-cluttered spaces with ease, adapting their flight and echolocation signal design to optimize information extracted from complex echo soundscapes (Falk et al., 2014, 2015; Hiryu et al., 2010; Petrites et al., 2009; Warnecke et al., 2016; Wheeler et al., 2016).

Most studies investigating the bat's behavior in complex environments have challenged the animal to maneuver around obstacles using uncontrolled echo-acoustic variables that are difficult to quantify across individual subjects and trials (e.g. Moss et al., 2014). Recently, we attempted to address this limitation by studying the bat's echolocation and flight behavior in an experimentally controlled corridor, which constrained the animal's flight trajectory and systematically manipulated echo-acoustic information (Warnecke et al., 2016). Specifically, we quantified the echolocating bat's flight trajectory and timing of biosonar signals in response to different echo flow patterns. Echo flow, cascades of several echoes arriving at the ears of the flying bat, varies with the animal's call rate, velocity, head aim, and distance to objects in the environment. In this previous study, big brown bats flew through a corridor built from individually moveable poles, while their flight trajectories and echolocation calls were recorded. The walls of the corridors were experimentally manipulated, with either dense or sparse spacing between poles, which returned different patterns of echo flow to the bat. When bats flew through a corridor with walls built from symmetrically spaced poles that returned *balanced* echo flow patterns from opposite sides, they centered their flight trajectory within the corridor. An *imbalance* of echo flow patterns, created by manipulating the pole-spacing on opposite corridor walls, caused bats to veer away from

the side of densely spaced poles and toward the side of sparsely spaced poles (Warnecke et al., 2016). These findings prompted us to investigate whether bats adjusted their flight paths in response to specific echo-acoustic cues like the timing of echo cascades, or to steer away from the side that returned more intense echoes, the densely spaced corridor side.

In the present study, we directly tested whether echo intensity guided the bat's flight path selection. To do so, we wrapped the poles of the densely spaced corridor side in either single or double layers of felt, which attenuated the intensity of echoes from adjacent poles on that side by about 3.5 dB per layer of felt (see Figure S2). If the big brown bat's flight deviation is largely influenced by the intensity of echoes returning to the bat from either corridor side, then our felt manipulation of the densely spaced poles should drive the bat's flight path to the center of the corridor or even reverse the flight path deviation towards the densely spaced side. If, however, the returning echo intensity is not a major contributing factor in the bat's flight deviation within the corridor, we would expect the bat to continue showing the same trajectory we previously observed (i.e. deviation towards the sparsely spaced corridor wall; Warnecke et al., 2016).

Another factor that might influence bat flight trajectory selection is the interval between echoes returning from sparse or dense pole spacing inside the echo flow corridor; the relative timing of which would be largely unaffected by a felt manipulation. We thus also investigated the bat auditory system's response to echo patterns returning from sparse and dense corridor walls. Specifically, we hypothesized that local auditory evoked activity in the big brown bat inferior colliculus (IC) would show distinct responses to echoes arriving from individual poles at sparse spacing, but not for echoes

arriving from individual poles at dense spacing.

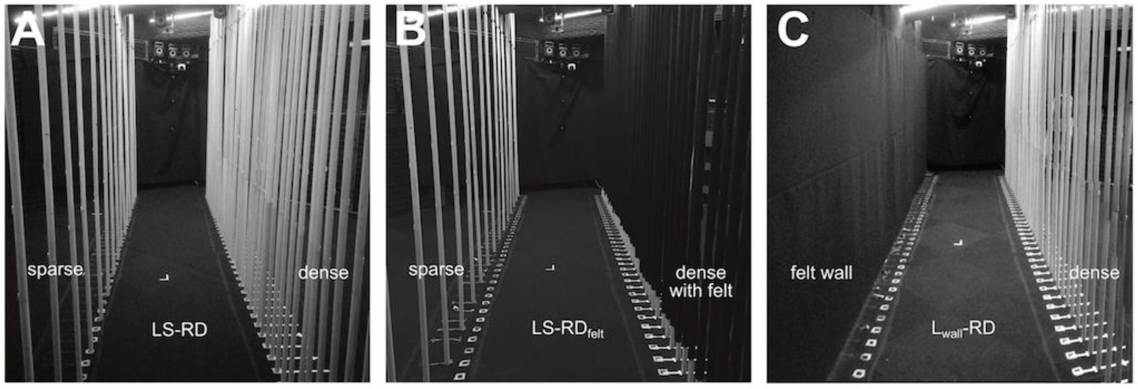
We conjecture that the auditory system of the echolocating big brown bat represents the echoes returning from each pole in the sparsely spaced corridor side as separate events in time and space, which the animal can use for flight guidance. By contrast, we propose that the interval between echoes from the poles in the densely spaced corridor wall return at such short time separations that the bat auditory system cannot resolve them, creating the representation of a single, smeared "wall of echoes," from which the bat steers away.

*Results*

In the present study, we investigated how different echo flow patterns influenced the flight path selection and auditory evoked responses in the big brown bat. Six echolocating big brown bats navigated through corridors of which the walls were built from wooden poles whose spacing could be manipulated to create different echo patterns (see Figure 1). To determine how flight and echolocation behaviors depended on changes in pole spacing (dense or sparse), baseline conditions presented bats with corridor walls whose poles were either symmetrically spaced on both sides, which created balanced echo flow patterns (LeftDense-RightDense, LeftSparse-RightSparse), or asymmetrically spaced across both sides, which created imbalanced echo flow patterns (LeftDense-RightSparse, LeftSparse-RightDense; Fig. 1A). To understand how flight and echolocation behaviors changed in response to changes in echo intensity, bats flew

through imbalanced corridors, with densely spaced poles wrapped in single or double layers of felt ($LD_{felt}$-RS, LS-$RD_{felt}$; Fig. 1B, LS-$RD_{2xfelt}$). We also tested flight behavior when one of the two pole walls was replaced with a felt curtain ($L_{wall}$-RS, $L_{wall}$-RD; Fig. 1C). Across all conditions, flight and echolocation behaviors were measured using IR tracking and a multi-channel sonar microphone array. To understand how the interval between separate echoes within echo flow cascades from the corridor influences the neural processing of echo cascades in the bat inferior colliculus (IC), we measured local evoked auditory activity in five awake, restrained big brown bats. Neural recordings were collected in response to the playback of natural echo recordings from the corridor, and artificial echo flow simulations. Below, we first outline the results of the flight behaviors across baseline and experimental conditions (see Figure 2). Subsequently, we describe the results of local evoked auditory responses (see Figure 3). Analysis of the echolocation behavior is presented in the Supplementary Information.

**Figure 3. 1. Photographs of selected conditions for behavioral data collection.**



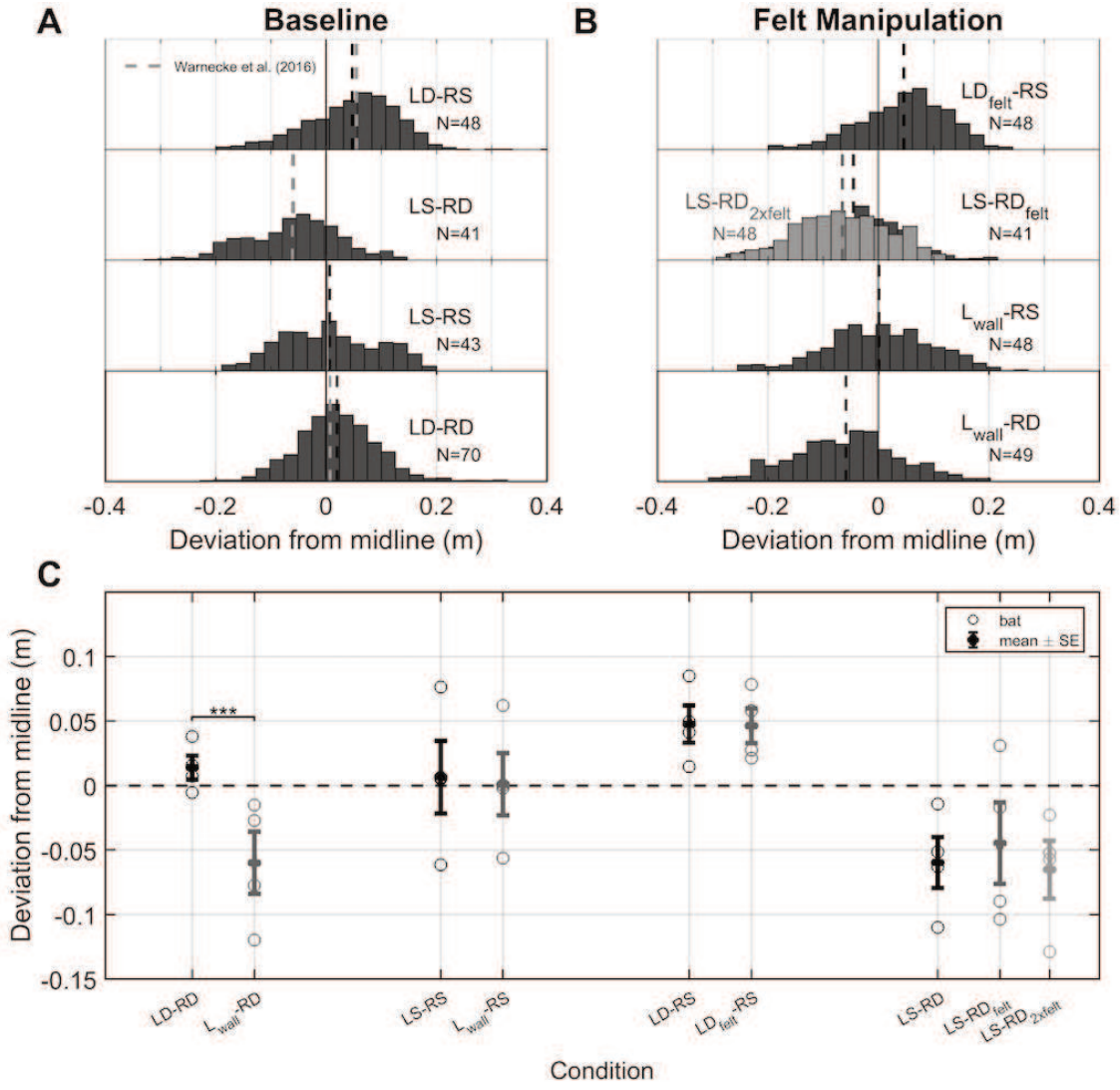

(A) Experimental setup of the LS-RD condition. (B) Experimental setup of the LS-$RD_{felt}$ condition, in which the densely spaced poles were wrapped in a single layer of sound-absorbing felt. (C) Experimental setup of the $L_{wall}$-RD condition, in which the left side of the corridor was created by hanging a single large piece of felt from a wire spun between two walls. Note that all photographs are showing only parts of the corridor, which did not fit into the picture in its entire length.

*Flight trajectories*

Figure 2 illustrates all bats' average flight deviation from the midline across baseline (Fig 2A) and experimental trials (Fig 2B). Positive numbers indicate deviations to the right, while negative numbers indicate deviations to the left side of the corridor. In baseline trials, bats centered their flight paths in balanced echo flow conditions (LD-RD: mean = 0.01 m, SEM = 0.02, LS-RS: mean = 0.006 m, SEM = 0.028), and changed their mean flight deviation toward the more sparsely spaced corridor wall in imbalanced echo flow conditions (LS-RD: mean = -0.06 m, SEM = 0.019; LD-RS: mean = 0.047 m, SEM = 0.014). Wrapping poles with felt to attenuate echoes did not change the flight trajectories from baseline: in experimental trials bats continued to deviate toward the sparsely spaced corridor walls (Fig 2B; LS-$RD_{felt}$: mean = -0.045 m, SEM = 0.03; $LD_{felt}$-RS: mean = 0.046, SEM = 0.013). Crucially, this behavior persisted, even when the densely spaced side was wrapped in two layers of felt, attenuating the echo intensity by about 7 dB (Fig 2B; LS-$RD_{2xfelt}$: mean = -0.065, SEM = 0.02; for information on the attenuation effect of felt application see Fig. S2). This indicates that the intensity of the returning echo cascade had no influence on the bats' flight path, compared to baseline trials. When bats flew in the corridor with one wall constructed from a single piece of felt, bats veered towards the felt wall when the opposite corridor side was comprised of densely spaced poles (Fig 2B, $L_{wall}$-RD: mean = 0.06 m, SEM = 0.02), and centered themselves when the opposite side was comprised of sparsely spaced poles (Fig 2B, $L_{wall}$-RS: mean = 0.001, SEM = 0.02). A repeated measures analysis between baseline and experimental data confirms that the change of deviation in LD-RD to $L_{wall}$-RD is the only significant change in the flight behavior data set (Fig 2C, black vs. grey data; $F_{3,12}$ = 6.33,

*p = 0.008*). Figures 2A,B plot histograms of the distributions of deviation across all bats and conditions; Figure 2C plots the average deviation (y-axis) per condition (x-axis; baseline: black, experimental: grey) for each bat (individual circles) and the mean ± standard error.

**Figure 3. 2. Behavioral flight adaptations to different echo flow conditions**




(A) Histograms showing deviation (x-axis) across baseline conditions (y-axis). Indicated are also the mean deviation for the current data (black dashed line), and for the data previously reported by Warnecke et al., (2016; grey dashed line). (B) Histograms showing deviation (x-axis) across experimental conditions (y-axis). Indicated is also the mean deviation (black dashed line), including data for LS-RD$_{2xfelt}$ (grey histograms and dashed line). (C) Distribution of mean deviation ± standard error (y-axis) across conditions (x-axis) per each individual bat (circles, N = 4) for baseline (black) and experimental (grey) conditions. Statistical significance is indicated with stars (*P = 0.008*).

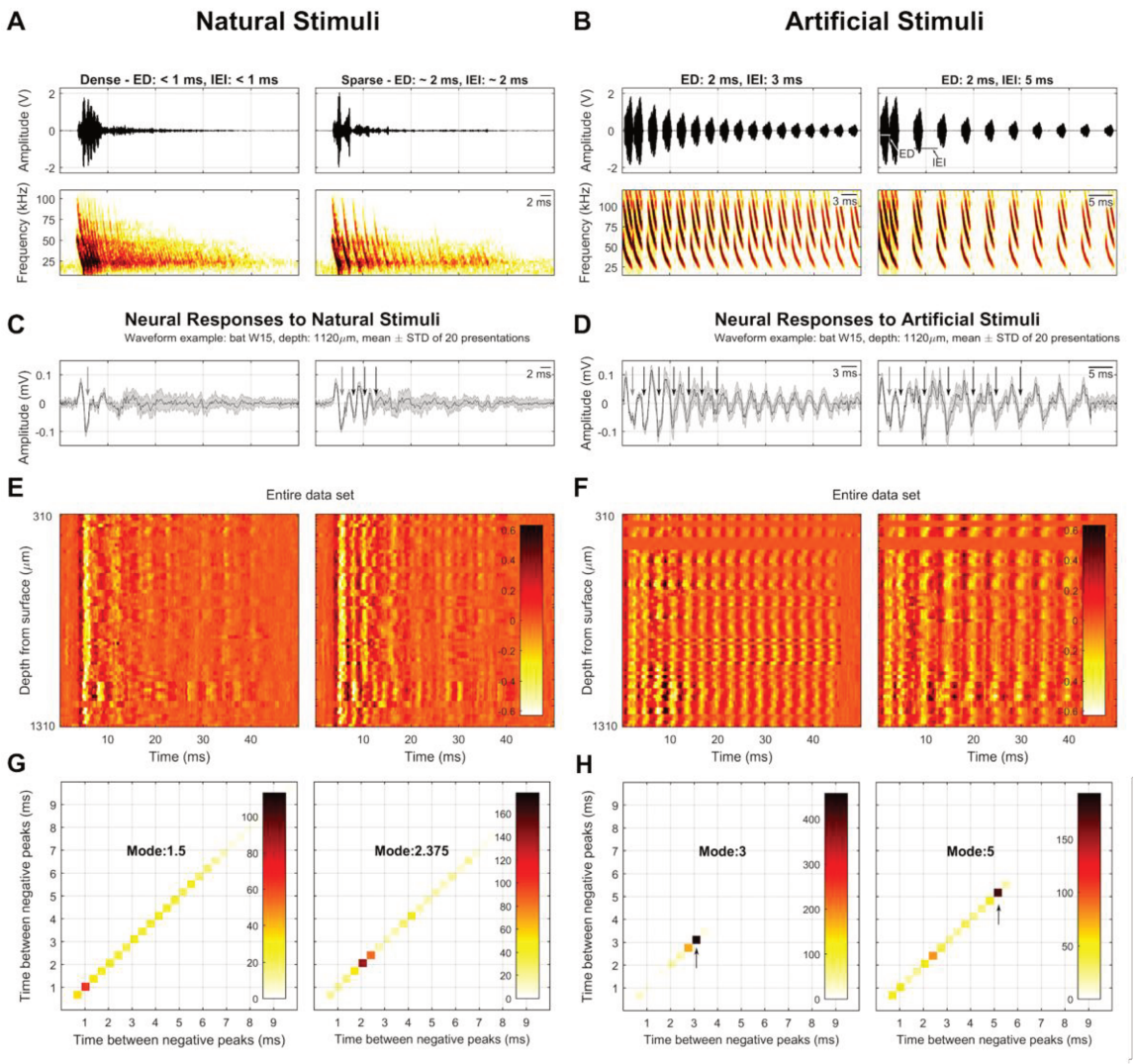
*Neural responses*

To investigate the bat auditory system's response to echo cascade patterns, we recorded local auditory evoked potentials in the bat IC. The acoustic stimuli presented during neural recordings were composed of two acoustic playbacks of echo cascades from dense (Fig. 3A, left) and sparse (Fig. 3A, right) corridor conditions, hereafter referred to as the "natural stimuli," and two simulated echo cascade stimuli, hereafter referred to as the "artificial stimuli." Intervals between echoes within cascades (inter-echo interval; IEI) were between 0.6 – 0.9 ms for the dense natural stimulus, and around 2 ms for the sparse natural stimulus. IEIs for the artificial stimuli were either 3 or 5 ms (for details see Methods). Averaged local evoked auditory activity to the respective stimuli are shown in Figures 3C – F. Figures 3C and 3D plot the averaged waveform acquired over 20 stimulus repetitions of an example response (black) ± standard deviation (grey) recorded in bat W15, at a depth of 1120 μm from the IC surface. In these examples, responses to the broadcast are distinct (grey arrow), and further evident for the subsequent echoes (black arrows) of the sparse natural stimulus, as well as the artificial stimuli. Peaks in the local evoked activity occurred at the inter-echo interval for each stimulus, even when separated by only 2 ms. Figures 3E,F illustrate heat plots for local evoked responses to natural (Fig 3E) or artificial (Fig 3F) stimuli, combining data from all bats across different depths (y-axis; 310 to 1310 μm) for the 50 ms duration of each acoustic stimulus (x-axis). The heat plots are normalized across natural and artificial stimulus conditions, with darker colors indicating positive peaks, and lighter colors indicating negative peaks. For both artificial stimuli (Fig 3F, IEI: 3 and 5 ms), a striped pattern at the 3 and 5 ms intervals for each row (depth and channel) is shown, indicating

that evoked responses across bats and recordings depths were modulated by the arrival time of mimicked echoes. Crucially, the striped response pattern appears only for the echoes in the sparse stimulus, which has an IEI of about 2 ms (Fig 3E, right), and is absent for the dense stimulus (Fig 3E, left). The neural data are aligned to the stimulus onset and thus do not reveal latency responses for each echo cascade across depths.

To determine whether the positive and negative peaks of the neural activity followed the temporal pattern of echo delays in each stimulus' IEI, Figures 3G,H plot the results of time delay measurements between negative peak responses to artificial and natural stimuli. Note that the IEI of the dense stimulus was too short to elicit any reliable neural responses. The number of responses occurring at a specific delay is indicated by shade, with darker shades showing an increase in responses. If neural responses are tied to the interval between echoes in each of the stimuli, the IEI, we would expect there to be darker shading at time delays corresponding to the stimulus-specific IEI. Figure 3H shows dark shading at around 3 and 5 ms (Fig 3H, left and right respectively, black arrows) corresponding to their respective IEI. Orange shading around 2 – 3 ms in Figure 3H (right) illustrates timing of evoked responses to the interval between broadcast and the first echo, which are separated by the echo delay (ED) of 2 ms (see Fig 3B, right; Fig 3D, right, grey arrow). Figure 3G (right) shows dark shading at 2 ms for the sparse acoustic stimulus (ED: ~ 2 ms, IEI: ~ 2 ms), while the shading is spread comparatively evenly between 1 to 6 ms of delays for the dense acoustic stimulus (Fig 3G, left, ED: <1 ms, IEI: < 1ms).

**Figure 3. 3. Stimuli and results of neural data collection.**




(A) Waveform (top) and spectrogram (bottom) plots of two Natural Stimuli recorded in the echo flow corridor (dense: left; sparse: right). Indicated are also the echo delay and inter-echo interval for each of these stimuli. (B) Waveform (top) and spectrogram (bottom) plots of two Artificial Stimuli created to mimic echo flow at different inter-echo intervals. Echo Delay (ED) and Inter-Echo Interval (IEI) are illustrated in the right top panel. (C) Waveform illustrates example trace of neural response recorded at a single depth for the two Natural Stimuli in (A). (D) Same as (C) but for Artificial Stimuli in (B). (E) Heat plot illustrating evoked response patterns across animals and depths for the two Natural Stimuli in (A). (F) same as (E) but for Artificial Stimuli in (B). (G) Cloud plots of delay calculations between peaks of neural responses for the two Natural Stimuli in (A). (H) Same as (G) but for Artificial Stimuli in (B).

*Discussion*

As animals navigate through their natural environment, they exploit sensory cues to adapt their locomotion in response to changes in the relative location of objects. In a manner similar to visually-guided animals' use of optic flow cues to steer locomotion, animals that rely largely on hearing may use acoustic, or echo flow cues to guide navigation (e.g. Kugler et al., 2016; Müller et al., 1999; Warnecke et al., 2016). For example, an echolocating bat that navigates through a densely-cluttered forest or flies along a forest edge, receives a cascade of echoes from trees at different distances for each sonar broadcast. Depending on the spacing between trees, the return of these echoes may arrive at short delay separations (for dense spacing of trees) or long delay separations (for sparse spacing of trees). The bat must thereby rapidly process and respond to echo cascades in complex environments and adjust its flight path to steer around obstacles. Here we demonstrate that the bat's flight path selection depends on the density of echoes returning from each sonar broadcast it produces.

To date, a few studies have investigated the effect of controlled echo cascades on bat sonar-guided navigation (e.g. Aharon et al., 2017; Petrites et al., 2009; Warnecke et al., 2016; Wheeler et al., 2016), and it is yet unclear what sensory cues drive flight trajectory selection. While most of these studies focused their analyses on echolocation behavior and not flight patterns, Aharon et al., (2017) tested if bats flying in long (~ 40 m) corridors, built from evenly spaced plastic poles on opposite walls, use the delay separation of echoes from individual poles for distance estimation. They trained *Pipistrellus kuhlii* to find a platform inside the corridor, and in test sessions determined

where the bat searched for an absent platform when the density of poles was experimentally manipulated, increased or decreased relative to training. They found that experimental manipulation of the pole spacing did not influence the bat's estimation of the platform location. Based on these findings, the authors propose that bats used internal self-motion cues or path integration, rather than echo returns, to estimate flight distance. This research does not, however, provide insight to the acoustic cues that guide flight trajectory selection in complex environments.

Recently, Warnecke et al. (2016) systematically varied the density of poles comprising corridor walls to investigate echo-guided flight and echolocation behavior. They found that when bats flew through corridors with symmetric spacing of poles on opposite walls, which returned balanced patterns of echo flow, animals centered their flight path in the corridor. However, when bats flew through corridors with asymmetric spacing of poles on opposite walls, which returned an imbalanced pattern of echo flow, animals deviated away from the dense pole/echo wall. While these results show that the flow of echoes returning from the corridor walls influences the bat's flight behavior, the intensity of echo returns from sparse/dense pole spacing could be a contributing factor. We hypothesized that greater relative echo intensity from the wall constructed from densely spaced poles influences the bat's flight path in the corridor. In the present study, we tested this hypothesis by combining different pole spacings with manipulations in wall echo intensity, by wrapping poles comprising the dense corridor wall with sound absorbing felt. Overall, the bat flight data show no difference in deviation from the midline of the corridor between baseline and experimental trials, in which poles were covered in felt to reduce the sound intensity of echo cascades (Fig. 2). This suggests that

echo intensity was not a major factor in the bat's deviation away from the densely spaced poles and towards the sparsely spaced corridor side. In other words, the findings do not support our hypothesis, and instead show that the flight paths of echolocating bats are not influenced by echo intensity of the corridor walls.

This result led us to hypothesize that differences in the timing of echo cascades from the dense and sparse pole spacing may influence bat flight behavior. To investigate how the bat processes the timing of echo cascades when navigating through the corridor, we recorded local auditory evoked activity from the big brown bat's inferior colliculus using stimuli representative of our echo flow soundscape.

Previously, Sanderson and Simmons (2000) recorded auditory evoked potentials in the IC of the anesthetized big brown bat, which was passively listening to two sonar stimuli mimicking a broadcast and an echo. They gradually increased the time delay between the two stimuli from 0 ms to 4 ms and showed that a discrete response to the second stimulus started to appear at inter-stimulus delays as small as 1.2 ms, though the evoked potential waveform did not return to full amplitude until the stimuli were separated by 2 ms. Given this result, we predicted that local auditory evoked activity in the bat IC would show distinct responses to echoes separated by intervals of 2 ms or longer, but that echoes at shorter intervals would not elicit discrete responses. The interval between echoes from the sparsely spaced poles is about 2 ms (Fig 3A, right), while the interval between echoes from the densely spaced poles is about 0.6 to 0.9 ms (Fig 3A, left). As such, we expected the local auditory evoked responses to the sparse pole echo recordings to show modulations for each echo, while we predicted such

discrete modulations would be absent in responses to echoes from the dense pole recordings.

At every recording site in the IC, both the auditory response waveform and the heat map, show closely spaced positive and negative peaks at intervals that match the IEI of the sparse echo flow stimulus (Fig 3A,C, right). Further, the neural responses to the artificial stimuli are also aligned with their inter-echo interval (Fig 3B,D), confirming that the response patterns seen in Fig 3C,D are linked to the patterns of echoes presented in each acoustic stimulus. The reliability of these modulations for each acoustic stimulus is also illustrated in Fig 3G,H, showing that the time delays between these responses matches the IEI of the stimulus (black arrows). In contrast, the waveform and heat map constructed of neural responses to the dense echo flow stimulus (Fig. 3A,C, left) do not align with arrival times of echoes in that condition.

Note that for all neural data the responses to the weaker, later echoes in the cascade show a slightly longer latency relative to the responses of the stimulus broadcast and the stronger, early echoes in the cascade, due to amplitude-latency-trading (Burkard and Moss, 1994; Ma and Suga, 2008; Pollak, 1988). This is especially pronounced for the neural responses to the natural stimuli, as the echoes in the cascade attenuate quickly over time. This may explain the slight overlap into longer delays for echo responses to the sparse stimulus shown in Fig 3G (right, black arrow). Additionally, the attenuation of the stimulus amplitude explains the rapid drop in amplitude of the local auditory evoked activity following the first echoes of the natural stimuli. The evoked response to the sparse natural stimulus sometimes extended to about 30 or 40 ms at shallow recording

depths (Fig 3E, right), but shortened to about 15 – 20 ms with increasing recording depths.

The differences in responses to the dense and sparse echo cascades suggest that the sonar receiver of the echolocating big brown bat may represent individual echoes returning from sparsely spaced poles as multiple discrete events, while it may represent echo cascades from the densely spaced poles as a single extended event that lasts over 35 – 40 ms. We thus conjecture that when the bat navigates through the imbalanced echo flow corridor, it deviates towards the sparsely spaced corridor side to "hear out" a pattern that it can use for sonar guidance. This pattern is discriminable in the echoes returning from the sparsely spaced corridor side, because the echoes return at inter-echo intervals of about 2 ms, which the bat auditory system can resolve. By contrast, echoes from the densely spaced corridor poles return at intervals of approximately 0.6 – 0.9 ms, likely too short for the local evoked activity in the auditory system to resolve (Sanderson and Simmons, 2000).

For each sonar broadcast, the echo pattern returning to the bat in the corridor is a complex sound wave containing echoes from both walls whose characteristics depend on the pole spacing. If the bat remains in the middle of the corridor, we hypothesize that "hearing out" an acoustic pattern that the bat can resolve and use for sonar guidance may be difficult, because acoustic scattering from both corridor walls contributes to complex spectro-temporal echo patterns. However, deviation away from the densely spaced corridor wall and steering toward the side that returns cascades of echoes at resolvable intervals (~ 2 ms or longer), increases the amplitude of echoes from that (sparse) side,

and thereby mitigates perceptual separation of sparse echoes from those of the opposite dense corridor wall.

How does the proposed representation of echo cascade patterns relate to the flight behavior across conditions in this study? When the bat navigates through the imbalanced corridor in the baseline condition (LeftSparse-RightDense, LeftDense-RightSparse), it can hear out a pattern, and thus deviates towards the side that returns echoes at intervals long enough to be represented as discrete events, the sparse side. By contrast, the bat deviates away from the side that returns echoes at shorter intervals, which may be represented as a single, smeared "wall of echoes." When the bat navigated through the imbalanced corridor in experimental trials with felt-wrapped poles on the dense side (LS-$RD_{felt}$, $LD_{felt}$-RS), it showed the same flight behavior as in the imbalanced baseline trials with bare poles (LD-RS, LD-RS). Note that wrapping the densely spaced poles with felt attenuates echoes by 3.5 dB (single layer) to 7 dB (double layer), compared to bare poles, but the relative delay between the attenuated echoes on either corridor side does not change.

We gain further insight to the acoustic cues guiding the bat's flight behavior from data in trials where we replaced the poles with a felt wall, which returns weak echoes following each sonar broadcast. When the bat navigated through the $L_{wall}$-RS corridor condition, it centered its flight path. However, when the bat navigated through the $L_{wall}$-RD corridor condition, it deviated away from the densely spaced pole wall, because echoes from this wall return at very short intervals, creating a smeared "wall of echoes." Importantly, it appears that it is not the felt wall attracting the bat toward that side in the $L_{wall}$-RD condition, but the close pole spacing of the dense wall driving the bat's *away*

from that corridor side. If the weak echoes that return from the felt wall reliably guided the bat's flight, the bat should also deviate away from the sparsely spaced poles in $L_{wall}$-RS. Since the bat centers its flight path in this corridor condition, we infer that the bat's behavior is not guided by the strength of echoes but instead on the time intervals between echoes from adjacent poles. The bat's flight path selection across corridor conditions is consistent with neurophysiological recordings showing discrete responses to sounds that are separated by a minimum of 2 ms (see Figures 2 and 3).

Neurophysiological studies in several bat species have measured single unit responses to broadcasts of natural echolocation sequences, which contained echoes from one or more objects (Bartenstein et al., 2014; Beetz et al., 2016, 2017; Greiter and Firzlaff, 2017; Sanderson and Simmons, 2000). For example, researchers presented the anesthetized FM bat *Phyllostomus discolor* with acoustic pulse-echo stimuli that simulated the bat's decreasing distance to one (Bartenstein et al., 2014) or two (Greiter and Firzlaff, 2017) objects, and characterized single unit responses in the auditory cortex. The results show changes in echo-delay (range) tuning with temporal-acoustic alignment of pulse-echo pairs, but do not address whether single echoes within larger echo cascades could be tracked.

Single unit responses to echolocation sequences containing up to three echoes have been characterized in the IC and auditory cortex of the anesthetized fruit bat *Carollia perspicillata* (Beetz et al., 2016, 2017). In these studies, acoustic stimuli contained call-echo segments of natural echolocation sequences that were recorded from a bat swinging on a pendulum, which passed by three objects. Inter-echo intervals of these stimuli were always larger than 2 ms. The results show that cortical neurons

responded reliably only to echoes from the closest object, and suppressed responses to echoes returning from more distant objects. This cortical suppression of single unit activity was absent in the inferior colliculus, and the authors suggested that parallel processing of multiple echoes at the level of the midbrain IC might drive cortical representation of the closer objects (Beetz et al., 2017). The neurophysiological data presented in our study further suggest that many objects at very close spacing may be represented as the closest object, or a single, extended object. Our work also provides the foundation to investigate neural representations of echo cascades in free-flying bats, to fully characterize responses to natural echo flow patterns induced by an animal's movement in the environment (see Kothari et al., 2018).

Several engineering initiatives have made use of sensory-guided navigation to control autonomous vehicles (Baker et al., 2014; Conte and Doherty, 2008; Smith et al., 2013; Steckel and Peremans, 2017; Strydom et al., 2014), or create devices to help visually-impaired individuals move safely within their environment (Filipe et al., 2012; Katzschmann et al., 2018; Lee and Medioni, 2011). While some of these systems use patterns of light, such as optic flow, to process information from the environment (Conte and Doherty, 2008; Strydom et al., 2014), recent work in sonar-based navigation has incorporated acoustic flow cues to automatically steer unmanned vehicles through complex corridors (Baker et al., 2014; Peremans and Steckel, 2014; Smith et al., 2014; Steckel and Peremans, 2017; Vanderelst et al., 2016). Most of the acoustic-based navigation devices have been tested in environments that contain large objects or flat surfaces, and it would be interesting to test the behavior of these systems in environments that create echo flow patterns similar to those presented here. Future experiments with

new corridor configurations, which also manipulate the pattern of pole spacing, can investigate sonar-guided path selection and trajectory planning. Building on these results can, in turn, inform the design of sonar-guided navigation systems for mobile vehicles that must operate in dark, GPS-denied environments.

## *Materials and Methods*

Experimental protocols were approved by Johns Hopkins University Institutional Animal Care and Use Committee. Detailed methods on behavioral testing procedures, as well as all acoustic stimuli used during neural recordings, neural recording procedures, and data analysis are presented in the Supplementary Information.

## *Supplementary Materials and Methods*

### *Animals*

In the present study, six wild-caught big brown bats (2 females), *E. fuscus*, served as subjects in behavioral experiments, and an additional five wild-caught big brown bats (5 females) were subjects in the neural recording experiments. The bats were not food-deprived and fed with mealworms (*Tenebrio molitor*) daily to maintain their individual weights between 13 to 16 g. All animals were maintained on a reversed 12-hour light-dark cycle in a colony room kept at 24 to 28 degrees C, with 40 to 50 % relative humidity. Bats in behavioral experiments were housed in one group cage. Animals used for neural recordings were kept in individual cages. The experimental procedures were

approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

*Behavioral Experimental Setup*

Experimental setup and paradigms are the same as previously described in Warnecke et al. (2016), but new conditions were added. Briefly, in a large carpeted flight room (6 x 7 x 2.5 m), a 620 cm long and 120 cm wide corridor was built from individually moveable wooden poles (2.5 cm diameter). The spacing between poles on the left and right corridor sides could be experimentally manipulated and several conditions with different pole spacings were tested in the experiment. Additionally, felt strips were wrapped around the wooden poles in some conditions to reduce their acoustic reflectivity (Fig S2); see photographs in Fig 1. The entrance to the corridor was shielded by a black felt curtain with an elliptic release hole cut out (31 x 38 cm) to prevent the bat from gaining information about the different wall configurations until it began its flight through the corridor on each trial. We ran four baseline conditions with different pole spacing: left dense, right dense (LD-RD), left sparse, right sparse (LS-RS), left sparse, right dense (LS-RD; Fig 1A), and left dense, right sparse (LD-RS). Additionally, we ran three experimental conditions in which we manipulated both the spacing and the reflectivity of poles: left dense wrapped in felt, right sparse ($LD_{felt}$-RS), left sparse, right dense wrapped in felt (LS-$RD_{felt}$; Fig 1B), and left sparse, right dense wrapped in two layers of felt (LS-$RD_{2xfelt}$). A single layer of felt attenuated the echo intensity by about 3.5 dB, a double layer of felt attenuated the echo intensity by about 6.9 dB relative to bare poles (Fig S2). We also collected data on two additional experimental conditions, in which the poles forming one wall of the corridor were replaced by a single large piece of

felt that hung from a wire spun between two walls: $L_{wall}$-RS, $L_{wall}$-RD (Fig 1C). For all conditions involving poles, dense spacing refers to a 12 cm gap between two poles, and sparse spacing refers to a 36 cm gap between two poles.

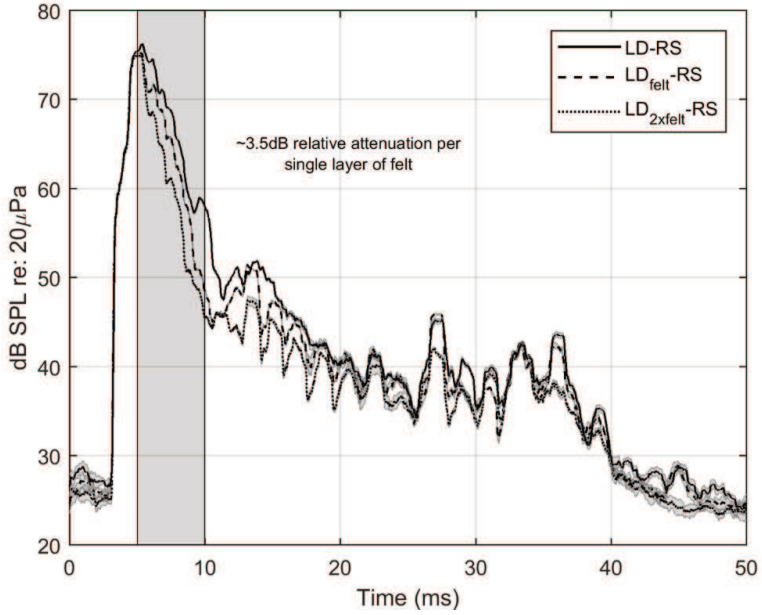
Prior to each experiment, an individual bat was removed from the cage and fit with a custom-built head marker, attached using water-soluble glue (Grimas Mastix Water Soluble, Heemstede, Holland). The head marker was triangular, with three small, 5 mm diameter reflective spheres glued to each corner. With a total weight of less than 0.1g, the marker was positioned between the bat's ears to track the animal's position within the corridor. The experiment started when the marker was securely attached to the bat's head and all recording systems were ready to collect data. Bats were released at ca. 150 to 100 cm distance from the corridor entrance curtain, and they entered the corridor by flying through the curtain hole. On each test day, bats flew through the corridor over at least nine trials. After data collection, the head marker was carefully removed, and the animal was returned to its cage.

Due to the duration it took to prepare each corridor setup, a single condition was tested on one day, but the order of spacing on the left or right, as well as the application of felt on the left or right side of the corridor poles was randomized. Each of the bats was tested in the same order and at approximately the same time of day. For each trial, three experimenters were present: one experimenter released the bat from behind the curtain and remained in that location. A second experimenter was responsible for catching the bat after a trial had ended and safely return it to the curtain-enclosed space. The third

experimenter recorded notes on every trial and triggered the audio and video recording systems (see below).

To restrict bats from using visual cues (Hope and Bhatnagar, 1979)(Hope and Bhatnagar, 1979) all data collection was done in a dark room that was solely illuminated with dim infrared light for motion-tracking detection of the reflective markers on the bat. Measurements of the light levels in the flight room at the beginning, middle and end of the corridor each revealed a light intensity of $<10^{-2}$ lux. Measurements were done using a spectrophotometer (GS-1500, Gamma Scientific, San Diego, USA) at experimental conditions.

**Figure 3. 4. S2 Attenuation of felt application**




Graph illustrates the effect of applying single (dashed black line) or double (dotted black line) felt layers to bare poles (solid black line) by plotting relative dB SPL values (y-axis) across time (x-axis) for three different conditions of the echo flow corridor (legend). Each line is an average of ten trials (black) ± standard deviation (grey) recorded 15 cm from the left corridor side of the condition indicated in the legend. Calculation of attenuation was restricted to time points from 5 to 10 ms in the recording (grey shading), because those time points contained echoes from the first meter beyond speaker and microphone locations, where little overlap with echoes from the opposite corridor side (sparse) had occurred. The mean attenuation at 5 to 10 ms in the recording was 3.46 dB SPL for LD-RS to LD$_{felt}$-RS, 6.94 dB SPL for LD-RS to LD$_{2xfelt}$-RS, and 3.48 dB SPL for LD$_{felt}$-RS to LD$_{2xfelt}$-RS. The attenuation of a single layer of felt was 3.47 dB SPL.

*Behavioral Data Recording*

For each trial, synchronized audio and motion-tracking data of the flying bat were collected. The bat's echolocation calls were recorded with 7 ultrasonic microphones (D500X external microphone, Pettersson Elektronik Uppsala, Sweden) and band-passed between 10 and 100 kHz (USBPBP-S2, Alligator Technologies CA, USA). Four microphones were mounted towards the end of the corridor, and three were mounted towards the beginning of the corridor. All audio data were sampled at 250 kHz (NI PXI board 6143). The bat's flight trajectory was acquired through 13 high-speed IR motion-capture cameras (Nexus, Vicon, Vicon Motion Systems Ltd., UK), mounted on the ceiling within the corridor. The Vicon camera system was calibrated on each test day and showed millimeter precision in localizing the reflective markers. The motion-capture system tracked the reflective spheres attached to each bat at 100 frames per second. Each test day, the motion-tracking program also recorded the position of the microphones, the location of the entrance hole, and the poles that made up the corridor walls. Every trial was manually triggered by an investigator after the bat had traversed the corridor at full length. Data acquired for the four seconds prior to the trigger were stored for off-line analysis.

*Behavioral Data Processing and Analysis*

Data were processed off-line using custom MATLAB programs (Mathworks, Natick, MA, USA) to digitally analyze the audio recordings of echolocation behavior and 3D flight trajectories of the bat.

For the audio analysis, we detected calls, extracted call start and end times, and calculated temporal characteristics. Across baseline and experimental trials, we collapsed

the conditions that create an imbalance of echo flow patterns (LS-RD, LD-RS) into one condition (S/D), as the pole patterns are identical with regard to their effect on echolocation variables. Main analyses focused on the average call duration, pulse interval and call rate across conditions.

Flight paths were reconstructed as 3D trajectories of each bat's navigational patterns in a given condition. These data were used to calculate the bat's 3D deviation from the midline of the corridor. Data points are calculated as distance from the end of the corridor, which has been defined as the plane created by the last poles on the left and right sides. To exclude data in which the bat first enters the corridor or may be planning its exit, data points at distances outside of 0.5 m from the start of the corridor and 1.5 m from the end of corridor were not included in the analysis. Only data collected from the middle portion (a total of 4 m) of the corridor were analyzed. We excluded trials that were classified as too short (3 m of flight or less), and collected at least 9 trials per bat per condition. For analysis of flight patterns, we calculated the mean 3D deviation for each 10 cm bin within the 4 m length of the middle portion of the corridor. These data points are plotted as a histogram distribution of deviation (x-axis) across conditions (y-axis) in Figure 2 for the baseline (A) and experimental (B) conditions. We also calculated the mean flight deviation per trial, for each bat (Fig 2C, circles) and each condition (Fig 2C, mean ± standard error). A total of 202 trials were analyzed for the baseline conditions (LS-RS, LD-RD, LS-RD, LD-RS), and a total of 186 trials were analyzed for the experimental conditions ($L_{wall}$-RS, $L_{wall}$-RD, LS-$RD_{felt}$, LS-$RD_{2xfelt}$, $LD_{felt}$-RS; see Fig 2A,B).

Statistical tests were performed using JMP (SAS). To evaluate whether flight

deviation differed between baseline and experimental trials, we used a repeated measures analysis with bat as a random factor. To evaluate the change of echolocation parameters (call rate, call duration, pulse interval) across appropriate variables of interest (baseline/experimental, condition, sonar sound group), we used a mixed model analysis in which we entered bat as a random factor and appropriate variables as fixed factors. If post-hoc testing was necessary, we used a Tukey's HSD.

Two of the six bats were excluded from behavioral data analysis: one bat never flew further than halfway down the corridor, and the other bat showed a persistent side bias toward the left in all conditions.

*Acoustic Stimuli in Neurophysiological Recordings*

To investigate local evoked neural activity to echo cascades from the different corridor conditions, we took acoustic echo recordings in the behavioral echo flow corridor setup, which later served as acoustic stimuli in neurophysiological experiments. We refer to these stimuli as the "Natural Stimuli." Acoustic stimuli were recorded inside the corridor at a 15 cm distance from the corridor wall by broadcasting a computer-generated 1.5 ms logarithmic FM sweep with two harmonics (FM1: 55 to 20 kHz, FM2: 110 to 40 kHz), and recording the echoes returning from the corridor poles. The sonar broadcast duration was chosen to match the shortest duration produced by bats flying through the corridor (see Results). Echo recordings were made using a set of seven ultrasonic microphones (D500X external microphone, Pettersson Elektronik Uppsala, Sweden) that were arranged in a hexagon and mounted immediately below an emitting loudspeaker (flat frequency response; Pioneer PT-R9). Both loudspeaker and

microphones were aimed straight along the corridor wall towards the end of the corridor. Echoes were sampled at 250 kHz (NI PXI board 6143, National Instruments, Austin, TX, USA), and subsequently band-pass filtered between 10 and 100 kHz (USBPBP-S2, Alligator Technologies CA, USA). For recordings of the sparse conditions, the pole spacing of the corridor wall was 36 cm, and for recordings of the dense conditions, the pole spacing of the corridor wall was 12 cm. We isolated a 50 ms recording of a broadcast and echo cascade for each condition (dense, sparse; Fig 3A) and subsequently used the isolated sound file as an acoustic stimulus for either sparse or dense conditions in the neural recordings. Figure 3A illustrates both the waveform (top) and spectrogram (bottom) of the natural stimuli. Indicated are also the echo delay (ED), which describes the delay from the beginning of the biosonar signal mimicking the broadcast to the beginning of the first returning echo, and the inter-echo interval (IEI), which describes the delay between the beginnings of the echoes in the returning cascade (see indications on Fig 3B, right). Both the ED and IEI were measured manually from the recordings. The smallest delay was ~ 0.6 ms in the natural dense stimulus.

We also created a set of computer-generated artificial stimuli which mimicked a broadcast and a cascade of echoes at regular delays (Fig 3B). We refer to these stimuli as the "Artificial Stimuli." The biosonar signal used in this stimulus set was previously recorded from a bat resting on a platform and emitting calls to detect an object in an otherwise empty room. This signal was matched in duration to the mean call duration emitted by bats during the flight behavior experiment (see Results), and repeated at decreasing amplitudes for 50 ms, to match the duration of the sound file for natural stimuli. Figure 4B illustrates both the waveform (top) and spectrogram (bottom) of these

stimuli. Two stimuli were created: Both had an echo delay (ED) of 2 ms, but their inter-echo interval (IEI) was chosen to be either 3 ms (Fig 3B, left) or 5 ms (Fig 3B, right).

All acoustic stimuli (Figs 3A,B) were each presented 20 times in random order, with a 300 ms inter-stimulus interval. To reduce the recording time, we changed the inter-stimulus interval to 200 ms for all recording sessions after the first bat's data collection.

*Electrophysiological Data Recordings*

All bats in the neural recordings were naïve to the stimulus and had not taken part in the behavioral data collection. All bats were first anaesthetized using 1-3% isofluorene gas. Under anesthesia, the skin and muscles on top of the head were retracted without damaging muscles controlling ear position. A head post was secured to the skull on the frontal midline using cyanoacrylate gel (Loctite 411, Henkel Corp., Dusseldorf, Germany). Animal Care protocols were followed following the surgery, and at least 2 days passed before neurophysiological recordings began.

Extracellular recordings were taken in awake, restrained animals, inside a sound-proof and electrically-shielded booth. Individually, bats were placed in a body mold made of plastic foam, and the head was tightly fixed by inserting the skull-attached head post into a metal holder. Every bat was used in multiple recording sessions, which never exceeded 3 hours. No pharmacological agents were administered during recordings sessions. Using skull and brain-surface landmarks, a small hole (~ 1 mm diameter) was carefully made over the inferior colliculus with a scalpel blade. Neuronal recordings were collected using silicon probes from Neuronexus (Ann Arbor, MI, USA; 1x16 arrangement; 50 μm spacing between recording sites, 75 μm probe shank thickness),

which permitted simultaneous data collection from the IC at depths spanning 800 μm. The probe was lowered into the brain through intact dura, orthogonal to the brain surface. Electrode penetrations were reconstructed histologically. Recording depths were measured using a hydraulic microdrive (Stoelting Co., Wood Dale, IL, USA) mounted on a micromanipulator. The brain surface was used as reference point (0 □m) for depth measurement, and the recording depths ranged from 300 to 1310 □m. A silver wire, placed 1-2 cm rostral from the recording probe and underneath the skin, was used as ground. Neuronal data acquisition was achieved using an OmniPlex D Neural Data Acquisition System recording system (Plexon, Inc., Dallas, TX, USA), at a sampling rate of 40 kHz per channel, and 16-bit precision. The acoustic stimuli presented to the bat were amplified (Krohn-Hite 7500, Krohn-Hite, Brockton, MA, USA) and broadcast at a D/A rate of 250 kHz (National Instruments card PXIe 6358), through a custom-built ultrasonic loudspeaker that was mounted 60 cm from the bat's ear, opposite the recording hemisphere. We compensated for the speaker's frequency response through digital filtering and subsequently obtained a flat frequency response (± 1 dB up to 110 kHz). The overall maximum output for each sound file was set at 70 dB SPL. Synchronization between the neural recordings and sound broadcasts was achieved with a TTL pulse output from the National Instrument card and recorded by one of the analog channels of the Plexon data acquisition system used for neural recording.

*Electrophysiological Data Processing*

Multi-unit recordings taken from the 16 channels of the silicon probe were processed to measure local evoked auditory activity to each of the acoustic stimuli. Data

collected in channels that picked up evoked responses to the stimuli were first filtered, and then averaged over 20 presentations for the entire 50 ms window of acoustic stimulus presentation. Both natural and artificial stimuli were filtered between 200 and 2000 Hz, yielding a 5 to 0.5 ms resolution, which was sufficient to detect responses induced by echoes at all EDs and IEIs of the presented stimuli. Two of the five bats were excluded from neural data analysis, because their neural recordings were noisy and no clear activity to artificial or natural stimuli could be measured.

Figure 3 (C,D) plots examples of the neural traces for a single bat, at a specific depth (see Results). After processing, data from the bats across different recording sessions were combined and normalized separately for the artificial or natural stimulus sets. The data were then plotted as a heat map of depth (y-axis) across time (x-axis) in Figure 3 (E,F) for each acoustic stimulus condition.

To extract the delays between neural response patterns, peak detection was performed on the filtered neural data using MATLAB; negative peaks smaller than -0.05 mV amplitude were picked up. We then calculated the time between peaks, and plotted the resulting times against each other for each condition of artificial or natural stimulus (Fig 3 G,H).

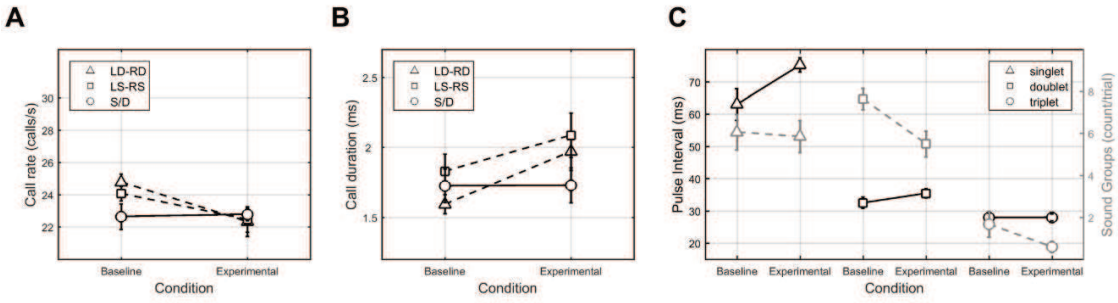
*Supplementary Results*

*Echolocation behavior*

We collapsed echolocation behavior measurements from the imbalanced corridor conditions (LS-RD, LD-RS) into one variable (S/D), as data showed that sonar call parameters did not differ in these two acoustically similar conditions. Overall, bats

emitted around 24 calls/s when flying in baseline trials, and around 22 calls/s when flying in experimental trials, in which dense poles were wrapped in felt (Figure S1, A). This difference was statistically significant ($F_{1,23} = 6.1$, $p = 0.021$). In all conditions, bats emitted very brief calls (~ 1.7 ms overall), with shortest calls observed in the most acoustically-cluttered condition, LD-RD (mean = 1.5 ms, SEM = 0.07), and longest calls observed in the least acoustically-cluttered condition, $L_{wall}$-RS (mean = 2.08 ms, SEM = 0.15, Fig S1, B). Overall, call durations were significantly shorter in baseline trials (mean = 1.71, SEM = 0.05), compared to experimental trials (mean = 1.87 ms, SEM = 0.07; $F_{2,23} = 11$, $p = 0.0004$). Post-hoc analyses revealed that the increase in call duration was driven by changes occurring between balanced conditions, LD-RD/$L_{wall}$-RD and LS-RS/$L_{wall}$-RS (Fig S1, B, dashed lines; LS-RS: t = -3.6, $p = 0.016$; LD-RD: t = -5.47, $p = 0.0002$, S/D: t = -0.01, $p = 1$). Call durations remained similar across imbalanced corridors in both baseline and experimental trials (Fig S1, B, solid line).

**Figure 3. 5. S1. Results of different echolocation behaviors.**




(A) Mean call rate (y-axis) ± standard error plotted across baseline and experimental trials (x-axis) for different conditions (balanced: dashed line; imbalanced: solid line, N = 3). (B) Mean call duration (y-axis) ± standard error plotted across baseline and experimental trials (x-axis) for different conditions (balanced: dashed line; imbalanced: solid line, N = 3). (C) Mean pulse interval ± standard error (black, left y-axis) and mean number of sonar sound groups ± standard error per trial (grey, right y-axis) plotted across baseline and experimental trials (x-axis), for different sonar sound groups (singlet: triangle, doublet: square, triplet: circle, N = 3).

In all corridor conditions, bats patterned their calls into packets of sonar sounds, which fell into one of three categories: grouping calls into sets of two (doublets) or three

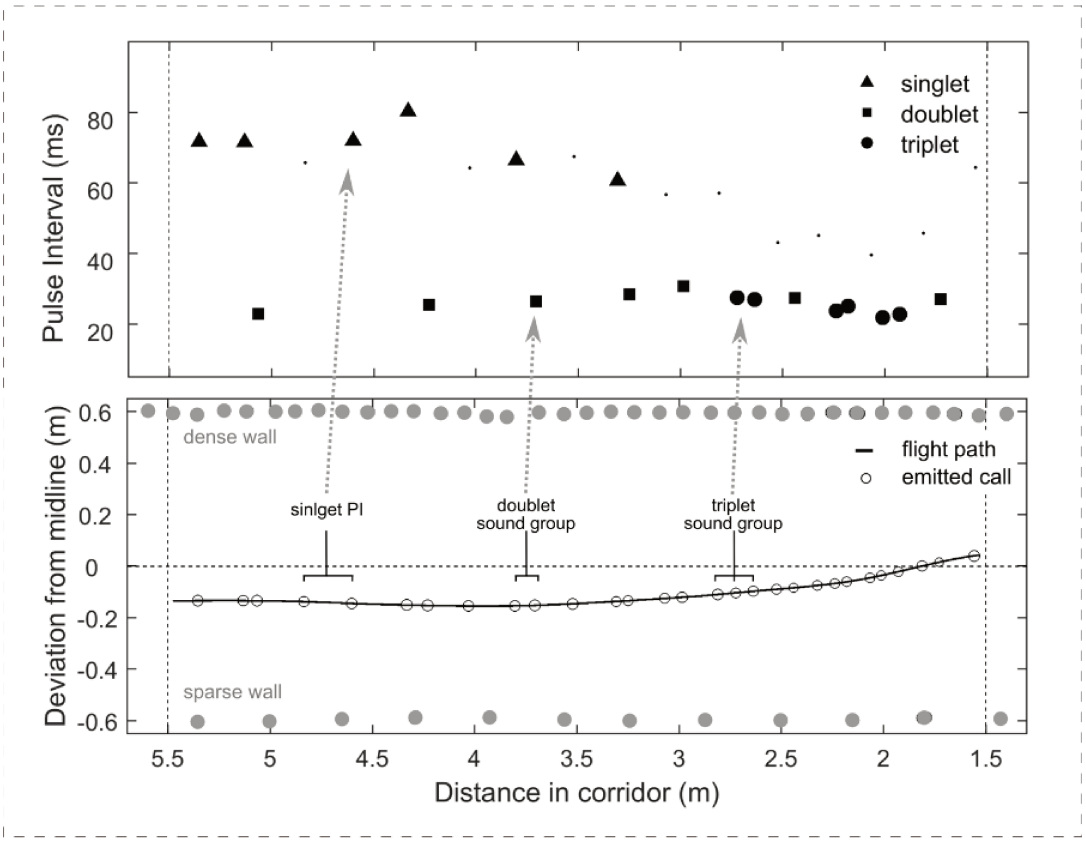
(triplets), or emitting calls that were not grouped (singlets) by definitions previously established (Kothari et al., 2014; Moss et al., 2006; Warnecke et al., 2016). Overall, bats emitted significantly fewer triplets than any other sound group (Fig 3C, grey; $F_{2,15} = 88.4$, $p < 0.0001$), with about 8% of sound groups falling into the triplet category. Most often, bats emitted calls that were classified as a doublet sound group (~ 48%), followed by ungrouped calls (singlets, ~ 43%). There were fewer sonar sound groups in experimental trials, compared to baseline ($F_{1,15} = 12.2$, $p = 0.0032$), and this difference was driven by a decrease in the occurrence of doublets (t = 3.74, $p = 0.019$; triplet: t = 1.78, $p = 0.5$, singlet: t = 0.54, $p = 0.99$). Across sonar sound groups, bats emitted calls at significantly different pulse intervals in baseline and experimental trials, generally increasing the intervals in the latter (Fig S1, C, black; $F_{1,15} = 18.9$, $p = 0.0006$). However, post-hoc analyses showed that this result is largely driven by the increase in pulse intervals between ungrouped, single calls (singlet: t = -5.88, $p = 0.0004$; doublet: t = 1.46, $p = 0.69$, triplet: t = -1.21, $p = 0.99$). Singlets were emitted at significantly longer PIs (mean = 68.4 ms, SEM = 1.6) than doublets (mean = 33.7 ms, SEM = 1.6, t = -22.4, $p < 0.0001$) or triplets (mean = 28.1 ms, SEM = 1.66; t = 26.17, $p < 0.0001$).

Throughout all conditions, bats mostly emitted short calls in doublet sound groups at an interval of 35 ms, which were flanked by longer, ~ 63 ms intervals (Fig S1, C, see Figure S3). This echolocation pattern was also reported by Warnecke et al. (2016), and suggests a strategy for echo processing. As is illustrated in Figure S1 A, each broadcast is followed by about 35 – 40 ms of echo cascades, and it is conceivable that the short interval between groups of sounds is utilized by the bat specifically to hear the entire echo cascade, before emitting the subsequent call. In fact, call intervals were rarely

shorter than ~ 35 ms, and if so, they occurred at the end of the corridor where the echo cascade was considerably shorter (Fig S3). Indeed, echo cascades could have only shortened in length, emphasizing that the bat should not experience any overlap between echo cascades in flight.

At a flight speed of about 3.5 m/s and emitting calls in groups of two at intervals of 30 – 35 ms, the bat travels approximately 10 – 12 cm between the onset of successive echo cascades. The separation between poles at sparse spacing is 36 cm, while the separation between poles at dense spacing is 12 cm. Thus, when flying past poles at sparse spacing, the bat could receive successive echo cascades from the same set of adjacent poles when emitting doublet call groups. By contrast, when flying past poles at dense spacing, the bat would receive successive echo cascades from different sets of poles from calls within doublet sound groups as it flies by adjacent poles. Small variations in pole positioning (see Figure S3) may thus allow the bat to track the position of individual poles across multiple sonar broadcasts when they are sparsely spaced within the corridor.

**Figure 3. 6. S3 Example of echolocation and flight behavior of one trial.**




Top: Plot of pulse interval (y-axis) across corridor length (x-axis) for calls emitted by a bat in an imbalanced baseline trial. Different sonar sound groups (singlet, doublet, triplet) are indicated by markers (see legend). Small black points are pulse intervals immediately following a sonar sound group. Black dashed lines indicate corridor limitations for analysis (see Methods). Bottom: Plot of flight deviation from the midline (y-axis) across corridor length (x-axis), for the same trial as in the top panel. Black solid line indicates x-y coordinates of bat flight path; white circles indicate locations at which the bat emitted a sonar call. Indicated are singlet, doublet and triplet sound group classifications, which relate to the data plotted in the top panel (grey dashed arrows). Grey filled circles represent x-y coordinates of poles at dense and sparse spacing for this trial. Black dashed vertical lines indicate corridor limitations for analysis (see Methods).

# Chapter 4

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# Behavioral adaptations to echo flow patterns across bat species

Completed Experiment Synopsis: A variety of echolocation signal types have evolved across different species of echolocating bats. For example, the Taiwanese leaf-nosed bat uses constant-frequency signals combined with an FM sweep (CF-FM), while the big brown bat, *Eptesicus fuscus*, modulates the structure of its frequency-modulated (FM) signals as it searches for and captures prey. In general, signals are suited to the bats' ecological niches and sonar-guided behaviors, therefore it is of interest to understand how different echolocating species adapt their behaviors in response to the environment. In this study, we quantified the flight and echolocation behavior of the Taiwanese leaf-nosed bat as it flew in an experimentally controlled corridor. Further, we compare the behavior of this bat to that of the big brown bat, which previously flew in the same corridor (Warnecke et al., 2016, Chapter 2, 3). Our results show that the two species differ significantly in the selection of their flight paths, and we propose that each species' natural habitat and signal design may contribute to their behavioral responses to echo cascades in the flight corridor. We also demonstrate a difference in echolocation call patterning between the two species, which may point towards different cues that each bat uses in order to track its location within an acoustically-complex environment.

**Title: Echo flow patterns drive different behaviors in bats using constant-frequency and frequency-modulated sonar signal**

Michaela Warnecke, Benjamin Falk and Cynthia F. Moss

In Review at *The Journal of the Acoustical Society of America*, April 2018

*Abstract*

In this study, we compared the echolocation and flight behaviors of the Taiwanese leaf-nosed bat, which uses constant-frequency (CF) biosonar signals, combined with a frequency-modulated (FM) sweep, with those of the big brown bat, which uses FM signals alone. The CF-FM bat *Hipposideros armiger terasensis* flew through a corridor bounded by vertical poles on either side, and we manipulated the inter-pole spacing of the walls to create different echo flow conditions. We analyzed the bat's flight trajectories and echolocation behaviors across corridor conditions. Similar to the big brown bat, the Taiwanese leaf-nosed bat centered its flight trajectory within the corridor when the pole spacing was the same on the two walls. However, the two species showed opposite flight behaviors when the pole spacing differed on the two walls: While the big brown bat deviated from the corridor center towards the wall with sparse pole spacing, the Taiwanese leaf-nosed bat deviated towards the wall with dense pole spacing. Further, in comparison to *E. fuscus*, *H. a. terasensis* utilized different echolocation patterns showing a prevalence of grouping sounds into clusters of three. We propose that the two species' distinct signal designs may contribute to their differences in flight behavior.

*Introduction*

Most diurnal animals, including humans, rely on vision to navigate. In contrast, echolocating bats, nocturnal mammals, have developed a specialized active listening system that allows them to represent their environment with sound (Griffin, 1958). Echolocating bats produce high frequency acoustic signals and process information contained in returning echoes to detect, localize and discriminate objects in their surroundings (Falk et al., 2011; Moss and Schnitzler, 1995; Simmons, 1973). Bats occupy many ecological niches around the world, and forage in environments as diverse as the desert and the tropics (Kunz and Fenton, 2005; Neuweiler, 2000), solving real-world scene analysis problems using different signal structures. These signals can be broadly categorized as constant-frequency (CF) and frequency-modulated (FM) sounds. In general, CF signals are typically long (6 to 100 ms), narrowband signals that span about 1-3 kHz, and have a high duty-cycle (Figure 1A, right; Moss and Schnitzler, 1995; Schnitzler and Kalko, 2001; Simmons and Stein, 1980). By contrast, FM signals are short (0.5 to 15 ms), mostly downward-sweeping sounds with a bandwidth of about 30 to 100 kHz, and have a low-duty cycle. Often, they contain more than one harmonic (Figure 1B, right).

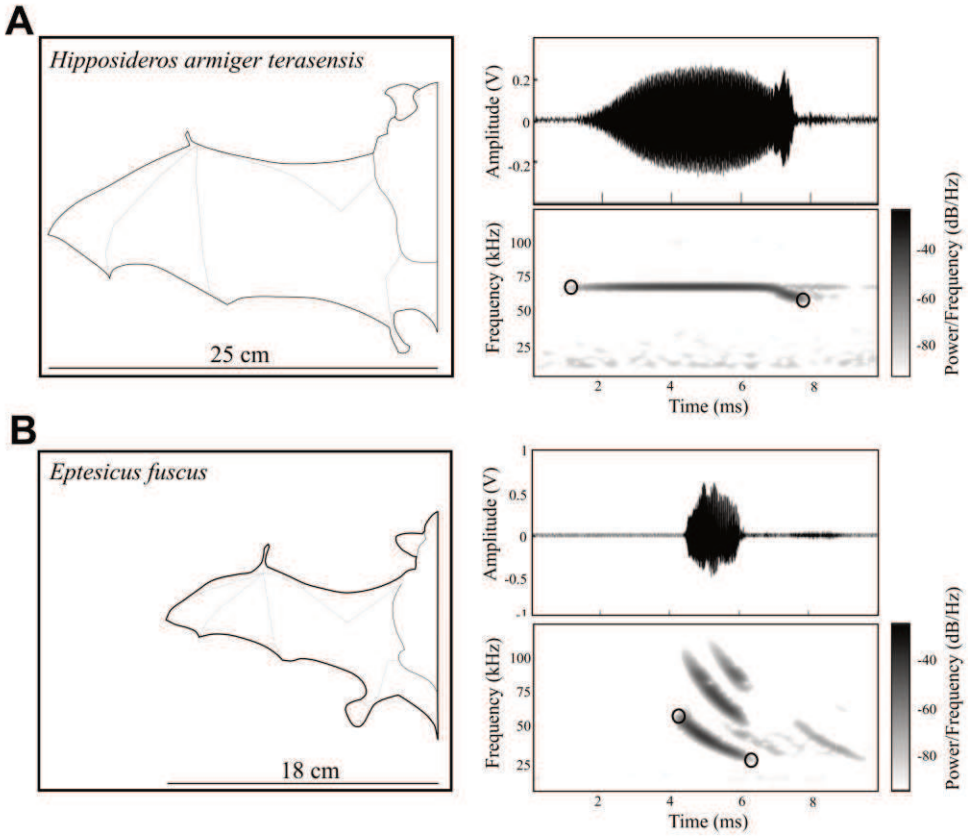
Bats that use broadband FM signals show high localization accuracy, particularly along the range axis, because each frequency in the signal provides a time marker for estimating echo arrival time (Simmons and Stein, 1980). By contrast, bats that use CF signals compensate for velocity-dependent Doppler shifts in returning echoes by lowering the frequency of their narrowband sonar emissions as they fly (Schnitzler, 1973). This

Doppler shift compensation (DSC) ensures that echoes return to the bat's sonar receiver at the frequency of maximum hearing sensitivity and frequency selectivity (Neuweiler, 1980; Neuweiler et al., 1980), and allow it to detect and discriminate fluttering insect prey in acoustically cluttered environments (von der Emde and Menne, 1989; von der Emde and Schnitzler, 1986, 1990; Kober and Schnitzler, 1990; Neuweiler, 1980; Schnitzler and Flieger, 1983; Trappe and Schnitzler, 1982). Previous work investigating the DSC behavior in some *Hipposideros* species raised the possibility that this bat's compensation is incomplete in comparison to that of other CF species (Habersetzer et al., 1984; Hiryu et al., 2006; Schuller, 1980). However, recent work suggests that the variable frequency shifts observed in *H. armiger* during flight may be explained by more variable emission frequency at rest across individual animals (Schoeppler et al., 2018). In other words, the DSC behavior of *H. a. terasensis* matches that of other CF species, when measurements take into account the variable resting frequencies of individual bats before each flight.

A variety of methods have been employed to investigate echolocation behavior and performance in bats that use FM and CF signals. For example, psychophysical studies have demonstrated the limits of distance, direction, size, shape, movement and texture discrimination in a variety of bat species (reviewed in Moss and Schnitzler, 1995; Wohlgemuth et al., 2016). Behavioral studies have also considered the contribution of the active control over the spectrum, timing and directional aim of sonar signals to the process of echolocation in different species of bats (von der Emde and Schnitzler, 1986; Gustafson and Schnitzler, 1979; Hiryu et al., 2007; Neuweiler et al., 1980; Schnitzler, 1973; Schnitzler and Flieger, 1983; Schoeppler et al., 2018; Surlykke et al., 2009b;

Surlykke and Moss, 2000; Trappe and Schnitzler, 1982). Additionally, recent studies show that bats using FM and CF signals adapt the timing between their sonar pulses (pulse interval, PI) to form sonar sound groups, in which the interval between sounds within a group is short, and is flanked by calls at longer intervals (Fujioka et al., 2014; Moss et al., 2006). The use of sonar sound groups is especially prominent in situations where bats would benefit from increased spatial resolution, such as foraging (Kothari et al., 2014) or navigating highly cluttered environments (Hiryu et al., 2008; Petrites et al., 2009; Sändig et al., 2014; Warnecke et al., 2016; Wheeler et al., 2016).

**Figure 4. 1. Comparison of *Hipposideros armiger terasensis* and *Eptesicus fuscus.***




(A) Sketched outline of *H. a. terasensis* body and wing to scale (left), typical CF-FM call emitted by *H. a. terasensis* (right). Top panel: oscillogram, Lower panel: spectrogram. (B) Sketched outline of *E. fuscus* body and wing to scale (left), typical FM call emitted by *E. fuscus* (right). Top panel: oscillogram, Lower panel: spectrogram. In both oscillograms, black circles indicate start- and end times typically extracted for audio analysis.

Although past work has informed our understanding of adaptive echolocation and flight behavior in diverse bat species, the acoustic basis of sonar-guided navigation in complex environments, where a single echolocation signal results in cascades of echoes, remains an open problem. Previously, Warnecke et al. (2016) investigated adaptations in flight and echolocation behavior in the FM bat, *Eptesicus fuscus*, under different conditions of controlled echo flow, in which cascades of echoes returning to the bat vary with its velocity, head aim, and distance to objects in the environment. In this study, FM bats flew through a corridor whose walls were built from individually moveable poles, and manipulations of the spacing between poles created different echo flow patterns for each emitted biosonar broadcast. The paper reported that bats adapted their flight paths and echolocation behavior in response to different spacing between poles on either corridor wall. For example, *E. fuscus* emitted shorter calls in the acoustically most cluttered condition, and typically arranged its calls into pairs of sonar sound groups when navigating the corridor (Warnecke et al., 2016).

Taiwanese leaf-nosed bats use CF-FM signals (Fig. 1A, right), and they commonly forage along forest edges and search for prey in confined environments like “corridors under the canopy” (Lee et al., 2012), a natural environment that mimics the experimental setup of the echo flow corridor introduced by Warnecke et al. (2016). In the present study, we studied this CF-FM bat species’ adaptations in flight and echolocation behaviors and compared data with that obtained from the big brown bat.

## *Materials and Methods*

### *Animals*

Three Taiwanese leaf-nosed bats, *H. a. terasensis*, served as subjects in this experiment after having been collected from the field in Taiwan and transported to Johns Hopkins University under CDC import permit 2015-03-146. The bats were fed with mealworms (*Tenebrio molitor*) daily to maintain their individual weights between 52 to 63 g. All animals were housed in a single colony room on a reversed 12-hour light/dark cycle, which was kept at 24 to 28 degrees C at 40 to 50 % relative humidity. The experimental procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

### *Experimental Paradigm*

The experimental setup and paradigms were the same as previously described in Warnecke et al. (2016). Briefly, in a large carpeted flight room (6 x 7 x 2.5 m) a 6.2 m long and 1.2 m wide corridor was built from individually moveable wooden poles (2.5 cm diameter). The spacing between poles on the left and right corridor sides could be experimentally manipulated and several conditions with different pole spacing were tested in the experiment. The opening to the corridor was lined with a black felt curtain that had an elliptic opening (31 x 38 cm) to prevent the bat from gaining information about the different wall configurations before it began its flight through the corridor on each trial. *H. a. terasensis* entered the experiment completely novice to the environment and had not undergone any prior training. We ran two baseline conditions in which the opposite walls of the corridor were symmetric: left dense spacing, right dense spacing

(LD-RD), or left sparse spacing, right sparse spacing (LS-RS). We also collected data in two test conditions in which the opposite walls of the corridor were asymmetric: left sparse spacing, right dense spacing (LS-RD), or left dense spacing, right sparse spacing (LD-RS). For all conditions, dense spacing refers to a 12 cm gap between two poles, and sparse spacing refers to a 36 cm gap between two poles. These gap measurements were chosen to be able to directly compare the data to a previously collected data set (Warnecke et al., 2016). To test if the effect of deviation changes for different corridor widths, we collected data for the LS-RD condition at corridor widths of 1.0 m, 1.2 m, and 1.5 m.

Prior to each experiment, the test bat was removed from its cage and water-soluble glue (Grimas Mastix Water Soluble, Heemstede, Holland) was used to attach a custom-built marker to the bat's head. The marker was shaped like a triangle and had three small (5 mm diameter) reflective spheres glued to each corner. At a total weight of less than 0.1 g, the marker was positioned between the bat's ears to track the animal's position during flight. The experiment started when the marker was securely attached to the bat's head, and all recording systems were ready to collect data. Bats were released at ca. 20 cm distance from the curtain, and they entered the corridor by flying through the elliptical opening. After collecting data over at least nine trials, the head and body markers were carefully removed, and the animal was returned to its cage.

Each day the bats were tested in the same order and at approximately the same time of day. Due to the time required to prepare each corridor setup, a single condition

was tested on each day. For each trial, three experimenters were present: one experimenter released the bat from behind the curtain and remained in that location. A second experimenter was responsible for catching the bat after a trial had ended and safely return it to the curtain-enclosed space. The third experimenter recorded notes on every trial and triggered the audio-video recording system for trial capture (see below).

All data collection was carried out in a room that was solely illuminated with dim infrared light for motion-tracking detection of the reflective markers on the bat (Hope and Bhatnagar, 1979). Measurements of the light levels in the flight room at different locations within the corridor each revealed a light intensity of $<10^{-2}$ lux. Measurements were made with a spectrophotometer (GS-1500, Gamma Scientific, San Diego, USA) under experimental conditions.

*Data Recording*

For each trial, synchronized audio and motion-tracking data of the flying bat were captured. Audio data were recorded using 7 ultrasonic microphones (6 D500X external microphones, Pettersson Elektronik Uppsala, Sweden, and one UltraSound Advice microphone, London, UK) bandpassed between 10 and 100 kHz (Alligator Technologies, CA, USA). Four microphones were mounted at the end of the corridor, and three were mounted in the beginning of the corridor. Microphones at the end of the corridor were used to collect the bat's calls, and to be able to extract temporal and spectral features of each call in combination with 3D flight track information, while microphones mounted at the beginning of the corridor more effectively sampled the echoes returning for each

emitted call. All audio data were sampled at 250 kHz (NI PXI board 6143). The bat's flight trajectory was recorded using 13 high-speed IR motion-capture cameras (Nexus, Vicon, Vicon Motion Systems Ltd., UK) mounted on the ceiling within the corridor. The motion-tracking system tracked the reflective spheres attached to each bat at 100 frames per second and allows for submillimeter precision. After all trials for the day were collected, the motion-tracking system also captured the position of the microphones, the location of the curtain entrance hole, and the poles that comprised the corridor walls. Every trial was manually triggered by an experimenter after the bat had traversed the full length of the corridor. Data acquired within 4 seconds prior to the trigger were stored for analysis.

*Data Processing and Analysis*

Motion-capture data were processed with custom-written MATLAB code to reconstruct 3D tracks of each bat's flight trajectories on a given day. In subsequent processing, we computed the bat's 3D deviation from the midline of the corridor. Data points are calculated with reference to the distance from the end of the corridor, which is defined as the plane created by the last poles on the left and right sides.

Echolocation calls produced by bats flying in the corridor were manually processed using custom-written MATLAB programs. For each trial, we extracted the call start and end times of each call that was emitted during the portion of the flight path (Fig. 1, black circles). With these parameters, we then calculated the call duration, duty cycle, call rate and pulse interval of each call.

To exclude positions in which the bat first entered the corridor or may have been

planning its exit, data points 1.5 m from the entrance and 1 m from the exit were excluded. Only audio and track data collected from the middle portion (a total of 4 m) of the corridor were analyzed. Trials in which the bats did not fly along at least ¾ of the length of the entire corridor were excluded. One bat's data was excluded from analysis in the LS-RS condition, because only three flights through the corridor met these requirements.

We used the statistical data analysis software JMP (SAS) to run statistical analyses on the data set. To evaluate whether the flight deviation differed across conditions, we used a mixed effect model analysis with condition (N = 4) as a fixed factor, and bat (N = 3) as a random factor. To evaluate whether the flight behavior of *Eptesicus fuscus* differed from that of *Hipposiderid armiger terasensis*, we calculated the difference between the mean deviation of each species per condition (see Figure 2A, dashed black lines), and tested whether that value differed significantly from zero. If both species showed similar flight path selections in a given condition, the difference between their mean deviations would be small. By contrast, if the species selected different flight paths in a given condition, the difference between their mean deviations would be large. We used a z-test to measure if the difference between mean deviations was different from zero.

For the analysis of audio data and flight speed we merged the conditions of different pole spacings, LD-RS and LS-RD, into a single variable "S/D", as we did not expect nor observe differences in echolocation behavior or flight speed depending on whether the densely-spaced poles were on the left or right side of the corridor. We used a

mixed effect model analysis to evaluate differences across conditions, with conditions (N = 3) as a fixed effect and bats (N = 3) as a random effect. After the initial analysis, if necessary, we performed a multiple comparison Tukey's HSD to evaluate (1) whether the bat's flight behavior differed between baseline (LD-RD and LS-RS) and test conditions (S/D), (2) whether echolocation parameters (duration, duty cycle, call rate) differed between conditions, and (3) whether pulse timings (PI, and number of sonar sound groups) differed between different sonar sound groups. A total of 124 trials (LD-RD: N = 35, LD-RS: N = 38, LS-RD: N = 29, LS-RS: N = 22) were analyzed.

*Results*

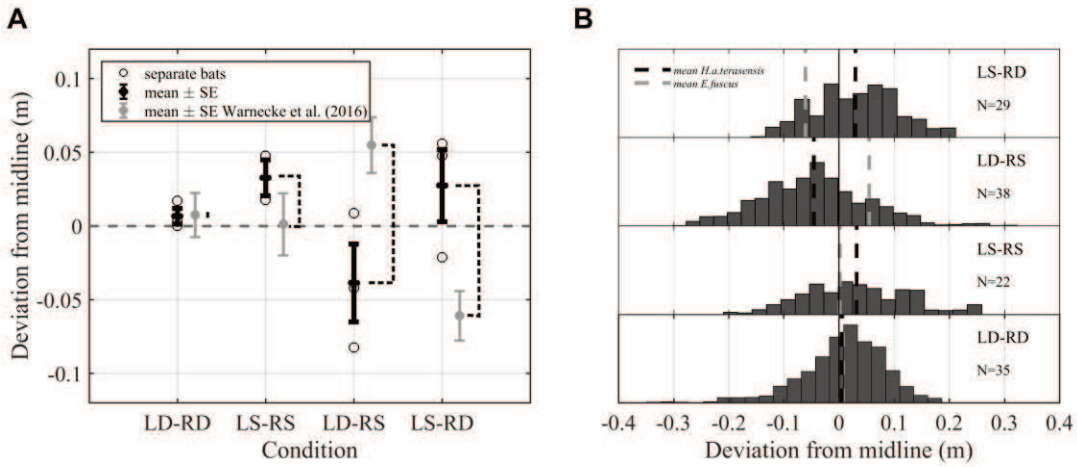
In this study, we tested whether different configurations of pole spacing along the sides of a corridor influenced the flight and echolocation patterns of the CF-FM bat, *Hipposideros armiger terasensis*. The flight data are displayed in histograms to represent the bat's deviation from the midline of the corridor: deviations toward the right are shown on a positive scale, whereas deviations toward the left are shown on a negative scale. Below, we first outline the flight behavior, and then describe the changes of echolocation parameters across conditions.

*Flight behavior*

The average deviation from the midline of the corridor for each of the four different conditions is shown in Figure 2. Figure 2A plots the mean deviation of each bat

(open circles), as well as the overall mean and standard error of deviation (black) for all bats across conditions (x-axis). Grey plots indicate the mean deviation of *E. fuscus* for the same condition (see Discussion; Warnecke et al., 2016). Figure 2B shows the distribution of deviations (x-axis) for each condition (y-axis), with the mean of each distribution indicated by a black dashed line for *H. a. terasensis*, and a grey dashed line for *E. fuscus* (Warnecke et al. 2016). Baseline conditions, LD-RD and LS-RS, present the bat with similar spacing of poles on the left and right corridor sides. When both sides are built from densely-spaced poles (LD-RD), *H. a. terasensis* centered its flight path around the midline and on average deviated about 0.0068 m (SE = 0.005) away from the midline toward the right side of the corridor. When presenting the bat with sparsely-spaced poles (LS-RS) on both corridor sides, the flight paths showed a broader distribution around the midline (Fig. 2B), with a slight shift to the corridor's right (mean = 0.031 m, SE = 0.013). Presenting the bat with different pole spacings on the corridor's left and right sides (LD-RS, LS-RD) caused *H. a. terasensis* to deviate slightly toward the more densely-spaced wall, and away from the more sparsely-spaced side (LD-RS: mean = -0.039 m, SE = 0.026; LS-RD: mean = 0.027 m, SE = 0.025). Our statistical analyses do not show a difference in the flight path distributions of deviations of *H. a. terasensis* from the midline across conditions ($F_{3,4} = 3.31$, $p = 0.116$).

**Figure 4. 2. Comparison of flight behavior across conditions.**




(A) Scatter plots of mean deviation per bat (open circles) across conditions (x-axis) are shown alongside plots of mean ± standard error per condition (black). Added in grey are the data points for the mean deviation of the same conditions collected by Warnecke et al., (2016) for the big brown bat. Dashed black lines indicate the magnitude of the difference value between means of each species per condition. (B) Histograms representing the deviation from the midline (x-axis) for all bats across four conditions (y-axis). Black dashed line indicates the mean deviation for *H. a. terasensis*. Grey dashed line indicates the mean deviation for the *E. fuscus* in the same conditions (Warnecke et al., 2016).

To evaluate whether the deviations of *H. a. terasensis* differs from that of *E. fuscus*, we calculated the difference between the mean deviation of the two species per condition (Fig. 2A, black dashed lines), and tested whether that difference is larger than zero. If the two species selected similar flight paths, the difference between their mean deviations would be close to zero (Fig. 2A, LD-RD, LS-RS), and if the two species selected different flight paths, the mean deviations would be greater than zero (Fig. 2A, LS-RD, LD-RS). Statistical analyses show that the difference between mean deviations in symmetric conditions do not differ from zero (LD-RD: difference = 0.0036, z = 0.13, $p = 0.44$; LS-RS: difference = 0.0287, z = 0.76, $p = 0.22$). However, in asymmetric conditions, the difference of mean deviations between *E. fuscus* and *H. a. terasensis*

flight trajectories is significantly larger than zero (LS-RD: difference = 0.088, z = 2.02, $p = 0.021$; LD-RS: difference = 0.0959, z = 1.92, $p = 0.027$).

*H. a. terasensis*' wingspan is almost thirty percent greater than that of *E. fuscus* (Fig. 1), and their flight path selection might have been impacted by the corridor width. To test whether the deviation from the midline was impacted by the width of the corridor, we also tested each bat in the LS-RD condition at three different corridor widths (1.0 m, 1.2 m, 1.5 m). Comparing mean flight deviation for this condition across the three corridor widths showed no significant difference of deviation toward the densely-spaced corridor side ($F_{2,4} = 4.3$, $p = 0.1$).

We investigated whether flight speed differed across pole density conditions, and did not find a difference in flight speed across conditions (Fig. 3A, grey; $F_{2,6} = 0.24$, $p = 0.78$). On average, bats flew at about 3.5 m/s, with slightly slower speeds in LD-RD (mean = 3.4 m/s, SE = 0.2) compared to LS-RS (mean = 3.53 m/s, SE = 0.08) or either of the test conditions (S/D: mean = 3.52 m/s, SE = 0.09).

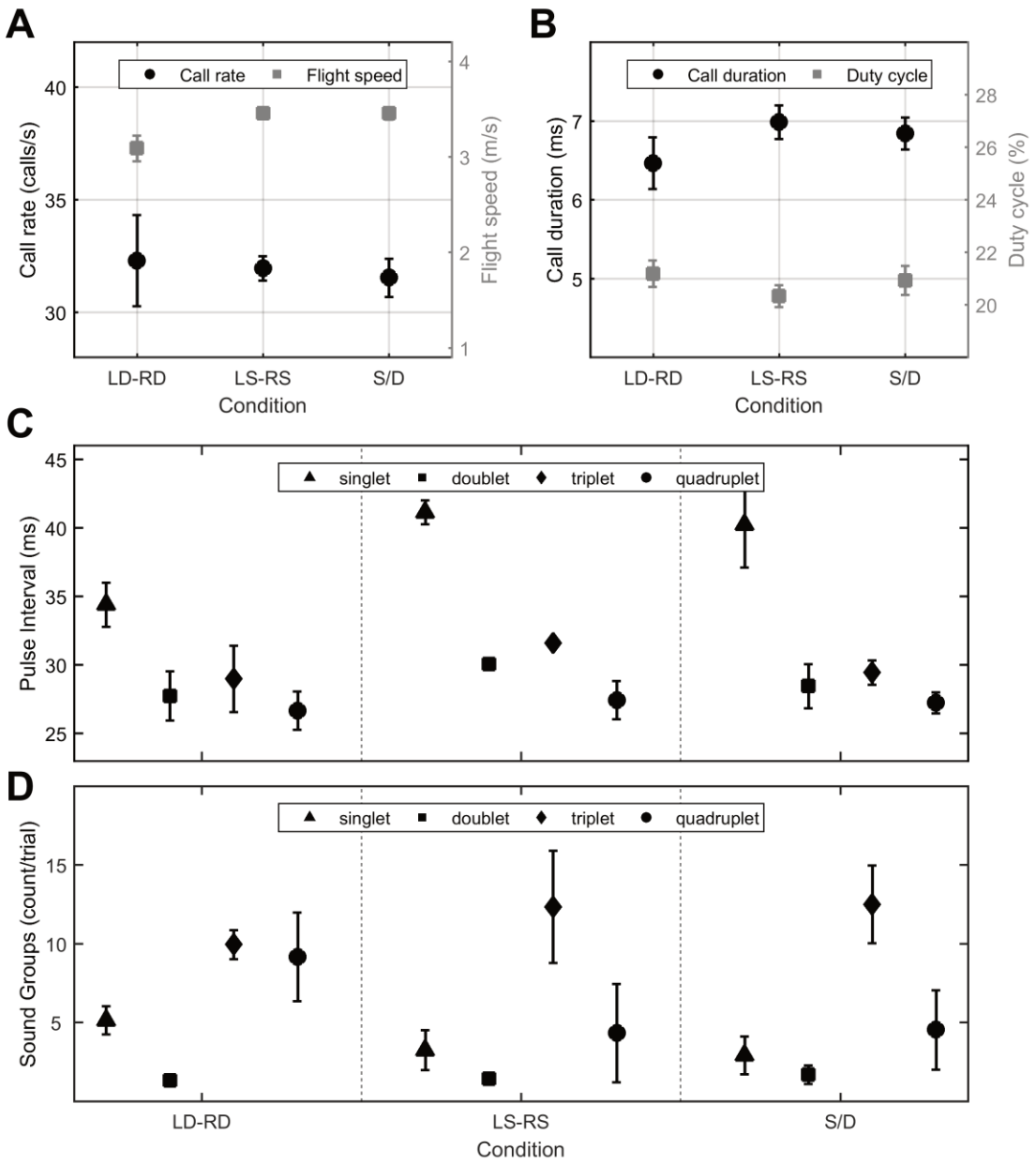
*Echolocation behavior*

Our results indicate that the bats did not alter their call rates across corridor conditions (Fig. 3A, black; $F_{2.6} = 0.005$, $p = 0.99$), and consistently emitted on average 31 calls per second (LD-RD: mean = 31.8 call/s, SE = 2.7; LS-RS: mean = 31.6 calls/s, SE = 0.76; S/D: 31.8 call/s, SE = 0.73).

Overall, *H. a. terasensis* emitted calls of typical length, about 6.8 ms long (Fig. 3B, black). We observed a strong tendency for the bat to produce shorter calls in LD-RD

compared to the other conditions (LS-RS, S/D; Fig. 3B; $F_{2,6} = 5.07$, $p = 0.054$; LD-RD: mean = 6.4 ms, SE = 3.2; LS-RS: mean = 6.9 ms, SE = 0.26; S/D: mean = 6.8 ms, SE = 0.13).

**Figure 4. 3. Echolocation behavior of *H. a. terasensis*.**




(A) Scatter plots indicating the call rate (left y-axis, black) and flight speed (right y-axis, grey) across conditions (x-axis) as mean ± standard error. (B) Scatter plot of call duration (left y-axis, black) and duty cycle (right y-axis, grey) across conditions (x-axis). (C) Scatter plots of pulse interval (y-axis) across conditions (x-axis) per sonar sound group type (legend). (D) Scatter plots of number of sonar sound group per trial (y-axis) across conditions (x-axis) for each sonar sound group type (legend).

Across all conditions, the bat oftentimes grouped its echolocation calls together into sonar sound groups. The bat's echolocation behavior fell into one of four categories: grouping calls into sets of two (doublets), three (triplets), or four (quadruplets) calls, or emitting sounds that were not grouped (singlets). Standards previously introduced to categorize sonar sound groups (e.g. Kothari et al., 2014; Warnecke et al., 2016) were slightly modified to match the patterning of pulse intervals used by *H. a. terasensis* into similar categories (decreased island criterion = 1.1, increased tolerance = 0.08, see Kothari et al., 2014). The prevalence of each of these four sound group categories was statistically different (Fig. 3D; $F_{3,36} = 19.1$, $p < 0.001$). Specifically, *H. a. terasensis* emitted triplet sonar sound groups significantly more often (Fig. 3D, diamond) than any other group (singlet – triplet: t= -5.7, $p < 0.0001$; doublet – triplet: t = -7.18, $p < 0.0001$; quadruplet – triplet: t = -3.96, $p = 0.0018$).

We also investigated the PI of each sonar sound group category and found a reliable difference in pulse interval (Fig. 3C; $F_{3,36} = 25.25$, $p > 0.0001$). Specifically, the PI of singlets was significantly longer (Fig. 3C, triangle) than that of any of the other sonar sound groups (singlet – doublet: t = -6.7, $p < 0.0001$; singlet – triplet: t = 5.4, $p < 0.0001$; singlet – quadruplet: t = -8.9, $p < 0.0001$).

We did not explicitly measure Doppler shift compensation for *H. a. terasensis*, because it was not necessary to monitor this behavior in the context of our study. Further, recent work suggests that the frequency shifts vary considerably between individuals and calls (Schoeppler et al., 2018), making the measurements of these behaviors challenging.

*Discussion*

Echolocating bats navigate in dark, acoustically complex surroundings with great agility and apparent ease (e.g. Griffin, 1958; Moss and Surlykke, 2001; Neuweiler, 1990; Simmons et al., 2001). In this study, we investigated flight and sonar adaptations of the echolocating Taiwanese leaf-nosed bat, *Hipposideros armiger terasensis*, in a confined, and reverberant corridor. We compare these adaptations to those observed in the big brown bat, *Eptesicus fuscus*, which navigated the same corridor (Warnecke et al., 2016), and propose that species differences in flight and echolocation behavior may stem from adaptations that *H. a. terasensis* and *E. fuscus* have evolved to their natural soundscape.

We predicted that *H. a. terasensis* would select different flight trajectories in corridors with asymmetric pole spacing on opposite walls, compared to the FM bat, *Eptesicus fuscus*, because the former commonly navigates in dense vegetation and might prefer to follow the wall with dense pole spacing. While *H. a. terasensis* does not show statistically reliable differences across conditions in their flight path selection, the data show a trend: when the bats flew through the corridor with the same approximate pole spacing on opposite walls, they centered their flight paths around the midline (Fig. 2B). In LS-RS, the distribution of flight trajectories is broader, which could be a result of the low number of bats in that condition (see Methods). When flying through the corridor in test conditions, the bats' mean deviation was toward the side with densely-spaced poles (Fig. 2B; black dashed lines). This trend is intriguing when one considers the flight paths taken by the big brown bat, *E. fuscus*, in the same corridor conditions, which show the

opposite pattern in the asymmetric wall corridor conditions (Fig. 2B; grey dashed lines). Indeed, statistical analyses show that flight path selection in the symmetric conditions do not differ between the two species, while in asymmetric conditions *E. fuscus* and *H. a. terasensis* do show significant differences.

*H. a. terasensis* is commonly found in cluttered environments, flying along forest edges, within the canopy, and foraging within dense vegetation (Lee et al., 2012). In these environments, each sonar call the bat produces results in multiple echoes returning to it from trees and shrubs at different distances. Thus, the CF-FM bat *H. a. terasensis* may commonly experience and use cascades of echoes for flight guidance in its natural habitat. The big brown bat, on the other hand, commonly forages in comparatively open spaces and over ponds, where echo cascades from vegetation may be rather sparse (Moss and Surlykke, 2010; Simmons et al., 2001; Surlykke et al., 2009b). We propose that each species' common foraging grounds and signal design could be a factor in their bias to deviate toward one side of the corridor in the asymmetric wall condition.

Echolocating bats adjust the timing of their sonar calls with respect to their environment and the task at hand (Hiryu et al., 2005; Kothari et al., 2014; Moss et al., 2006; Petrites et al., 2009; Schnitzler and Kalko, 2001a; Warnecke et al., 2016). In all conditions tested in this study, we observed that *H. a. terasensis* grouped its calls into clusters of sonar sound groups, as previously described in other bat studies (Kothari et al., 2014; Moss et al., 2006; Wheeler et al., 2016). Schoeppler et al. (2018) recorded *H. armiger* calls at rest and during flight using wall-mounted microphones, while Hiryu et al. (2005, 2008) recorded echolocation calls of the same species while carrying a custom-made telemetry microphone. Both studies reported sonar sound group production in bats

engaged in the search and approach phases and preparing to land (Hiryu et al., 2005, 2008; Schoeppler et al., 2018). This observation is consistent with previous reports of sonar sound group production patterns by the big brown bat in situations which demand high special localization accuracy (Kothari et al., 2014; Moss et al., 2006; Sändig et al., 2014; Warnecke et al., 2016 and Wheeler et al., 2016).

Audio analysis of echolocation behavior of bats in the present study revealed that *H. a. terasensis* more commonly produced sonar call triplets (Fig. 3D, diamonds), compared to other sound groups. Interestingly, in the acoustically most cluttered condition (LD-RD), animals also produced an increased number of quadruplets (circles). This differed from the other conditions (LS-RS and S/D), in which bats produced triplets more often than all other sound group categories, and suggests that the bat's production of triplets and quadruplets in the most cluttered corridor condition might be helpful in navigating through this complex soundscape.

In this context, it is noteworthy that the overall call rate of *H. a. terasensis* does not differ across corridor conditions (Fig. 3A, black), showing that the bats re-arranged the temporal patterning of calls to create triplets and quadruplets, rather than just "adding" or "deleting" calls to create a different sound group. Further, this implies that the use of higher order sound groups, i.e. triplets or quadruplets, may yield a sharper representation of the soundscape, and therefore support navigation through the acoustically most complex condition.

The prevalent use of sonar call triplets by *H. a. terasensis* contrasts with the big brown bat's prevalent use of doublets, followed by singlets. In fact, *E. fuscus* rarely made use of triplets or quadruplets (Warnecke et al., 2016). In short, while the Taiwanese leaf-

nosed bat most often used triplets, followed by quadruplets to navigate any echo flow condition, the big brown bat used those two sound groups the least, and relied on doublets and singlets. By emitting higher order sound groups, the Taiwanese leaf-nosed bat receives multiple "snapshots" of the environment as it flies by the poles. This may aid the bat in representing its soundscape.

When *H. a. terasensis* produced singlets, the PI was about 37 ms, which is considerably longer than any other sound group PI (~ 29 ms). At the entrance of the corridor the bat would receive a 30 – 40 ms long cascade of echoes of decreasing in amplitude. As such, the singlet PI would allow for echoes of the entire corridor to be received, before a subsequent call was emitted. At shorter intervals, like those in doublet, triplet or quadruplet sound groups, the bat would have been emitting a new call when echoes from the previous sonar emission were still arriving. We propose that the longer PI of singlets may have served as a "break" from rapid processing of echo information that occurred when emitting calls at shorter intervals (i.e. doublet, triplet and quadruplet PIs).

Differences in the interval and duration of sonar signals produced by *E. fuscus* and *H. a. terasensis* yields species differences in sonar call duty cycle. The big brown bats' calls are considerably shorter (~ 2 ms) than those of the Taiwanese leaf-nosed bat (~ 6.8 ms). These differences in duration, coupled with the prevalence of call triplets produced by *H. a. terasensis*, yield sonar duty cycles in this species that are several times higher than those of *E. fuscus*. (*H. a. t.*: ~ 18 – 22%, see Fig. 3B, grey; *E. f.*: 3 – 5%). The differences in duty cycle will dramatically influence the echo patterns used by the two species to represent the corridor.

Echolocating bats using CF-FM biosonar signals typically lower their outgoing call frequency to compensate for velocity-induced Doppler shifts and control the frequency of returning echoes to match their most sensitive region of their hearing (Schnitzler, 1973; Schoeppler et al., 2018). In the echo flow corridor, *H. a. terasensis* received a cascade of echoes from each sonar emission, which were Doppler shifted relative to the frequency of the bat's outgoing call. As *H. a. terasensis* flew in the corridor, its Doppler shift compensation would differentially affect the echo frequency of poles at different directions and distances (see Müller and Schnitzler, 2000). The bat may use Doppler echo flow patterns to monitor its position within the corridor. By contrast, *Eptesicus fuscus'* broadband hyperbolic FM sweep is largely Doppler-tolerant (Altes and Titlebaum, 1970), and this bat may use other echo cues to track its location within the corridor, such as arrival time of sonar returns embedded in the echo cascade.

In summary, we compared the echolocation and flight behavior of the CF-FM bat, *H. a. terasensis,* with that of the FM bat, *E. fuscus,* in an echo-flow corridor and quantified differences in the two species' behaviors. This study revealed that a corridor returning asymmetric echo flow patterns from opposite walls resulted in significantly different flight path selection and adaptive echolocation behavior in the two species. These findings may arise from the distinct echolocation call designs of the two species, and future work may elucidate differences in echo flow processing by FM and CF-FM bats.

# Chapter 5

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# A bat Cocktail-Party: Does silent behavior occur in clutter?

Completed Experiment Synopsis: Echolocating bats commonly forage among conspecifics, and despite potentially experiencing large amounts of overlap between calls from different individuals and echoes, these bats easily take prey on the wing. Previous work suggested that bats will sometimes cease to echolocate, i.e. produce "silent behavior" when they compete for a single prey item in open spaces (Chiu et al., 2008). Here, we extend that work by evaluating silent behavior in cluttered environments. We hypothesized that bats would reduce the amount of silent behavior in cluttered environments, compared to open spaces, as there is a greater need to constantly monitor the environment more closely. We did *not* find any evidence for silent behavior in *either* open or cluttered environments. Additional analyses revealed differences in flight behavior and gender composition of the bats run in the present study and those run by Chiu et al. (2008), and we propose that these factors may have influenced the production of silent behavior.

**Title: Active listening in a bat cocktail party: Adaptive echolocation and flight behaviors of big brown bats, *Eptesicus fuscus*, foraging in a cluttered acoustic environment**

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*Abstract*

In their natural environment, big brown bats forage for small insects in open spaces, as well as in vegetation and in the presence of acoustic clutter. While searching and hunting for prey, bats experience sonar interference, not only from densely cluttered environments, but also from calls of conspecifics foraging in close proximity. Previous work has shown that when two bats compete for a single prey item in a relatively open environment, one of the bats may go silent for extended periods of time (Chiu et al. 2008), which can serve to minimize sonar interference between conspecifics. Additionally, pairs of big brown bats have been shown to adjust frequency characteristics of their vocalizations to avoid acoustic interference in echo processing (Chiu et al., 2009). In this study, we extended previous work by examining how the presence of conspecifics *and* environmental clutter influence the bat's echolocation behavior. By recording multichannel audio and video data of bats engaged in insect capture in open and cluttered spaces, we quantified the bats' vocal and flight behaviors. Big brown bats flew individually and in pairs in an open and cluttered room, and the results of this study shed light on the different strategies that this species employs to negotiate a complex and dynamic environment.

*Introduction*

Many mammals rely on their visual systems to orient, navigate and forage. However, some species, like bats, dolphins and whales, have evolved specializations to actively probe their environments with sound: Echolocating animals send out sonar broadcasts and listen to returning echoes to perceive objects in their immediate surroundings (Griffin, 1958). They navigate and forage within a "bat cocktail party," so to speak, as many animals echolocate in the presence of other bats, each producing calls and listening to echoes, which they must sort from the signals of nearby conspecifics.

*Eptesicus fuscus*, the big brown bat, uses ultrasonic vocalizations to navigate and forage insects in its environment (Griffin, 1958; Surlykke and Moss, 2000). This bat's sound emissions are frequency-modulated (FM), downward-sweeping echolocation calls containing several harmonics in the range of approximately 25 to 130 kHz, which last between ~0.5 and 15 ms (Simmons, 1979; Surlykke and Moss, 2000). Call duration and the time between each emission (pulse-interval, PI) shorten in length as the bat searches for targets, approaches, and finally captures prey (Figure 1A; Simmons, 1979). Calls emitted during prey capture are referred to as the "terminal buzz" which ends in "buzz 2," where big brown bat pulse interval (PI) is ~6 ms and call duration is ~0.5 ms (Surlykke and Moss, 2000). Each broadcast echolocation call contains two prominent harmonics: FM1 sweeps from about 65 to 25 kHz, and FM2 sweeps from 130 to 50 kHz (Figure 1B) (Simmons, 1979, Moss and Schnitzler, 1989; Saillant et al., 2007 ; Ulanovsky and Moss, 2008, Surlykke et al., 2009, Moss and Surlykke, 2010). The broadband echolocation signals used by *E. fuscus* support accurate sonar localization in azimuth (Simmons et al.

1983), elevation (Lawrence and Simmons, 1982) and range (Simmons, 1973). To estimate the distance to a given object, the bat relies on the time delay between the emission of a call and the reception of an echo (Simmons, 1973, Simmons, 1989).

The horizontal sonar beam emitted by *E. fuscus* is spatially broad and can potentially ensonify not just targets of interest in its path, but also surrounding acoustic clutter, such as trees, branches, and leaves, as it spans about +/-70 degrees at frequencies contained in FM1, and about +/-40 degrees at frequencies in FM2 (Aytekin et al., 2004; Hartley and Suthers, 1989).

While navigating in its environment, the echolocating bat not only needs to localize and identify different targets (Simmons and Vernon, 1971 ; Moss and Schnitzler, 1995), but also discriminate them from background clutter (Simmons, 1973; Bates et al., 2011), such as trees, branches, conspecifics or water surfaces (Simmons, 1973; Chiu et al., 2009; Surlykke et al., 2009; Bates et al., 2011; Greif and Siemers, 2010). For the bat, echo analysis of its surrounding scene involves the segregation and integration of echo cascades arriving from objects at different locations (Moss and Surlykke, 2001; 2010). Auditory scene analysis for an echolocating bat is further complicated by the sonar signals of nearby conspecifics.

When approaching a target, *E. fuscus* steers its beam toward a selected prey item and eventually aims its broadcast at the target with an accuracy of 3 degrees during the final capture phase (Ghose and Moss, 2003). While the bat closely monitors the target's position, successful foraging also depends on minimizing sonar interference from clutter echoes and calls from other nearby bats. It has been suggested that bats attempt to reduce acoustic interference, i.e. background noise, and calls or echoes from nearby conspecifics

by either ceasing to echolocate and potentially "eavesdrop" on another bat's calls (Balcombe and Fenton, 1988; Barclay, 1982; Chiu et al., 2008; Gillam and McCracken, 2007) or changing characteristics of emissions such that calls and echoes can be distinguished from those produced by nearby conspecifics (Chiu et al., 2009).

Chiu et al. (2009) reported that the big brown bat shows adaptive spectral changes in its vocalizations when paired with a conspecific in a competitive prey capture situation. The researchers found that (1) as the spatial position of two bats decreased, the difference in their spectral call features increased, and (2) paired bats with similar baseline spectral call characteristics showed larger frequency adjustments compared to those with distinct baseline call designs.

Adjusting sonar call characteristics in the presence of conspecifics is an adaptive strategy for situations in which the bat needs to avoid interference from competing acoustic signals, and must also accurately search for and capture prey by processing target echo information. By changing spectral parameters of its echolocation signals, the bat continues to receive updates about the environment and its location in relation to a selected target, and at the same time it is able to match emission-echo pairs to distinguish its signals from those of another bat that may be broadcasting its sonar signals in close proximity.

Chiu et al. (2008) reported that pairs of flying big brown bats also exhibit silent behavior when competing for a single prey item. Remarkably, the researchers found that 40% of the time when the inter-bat separation was 1 m or less, one bat would stop echolocating for a minimum of 200 ms. This "silent behavior" persisted for flight trajectories covering between about 60 cm (200 ms) out to 8 m (2.55 s) and in one bat

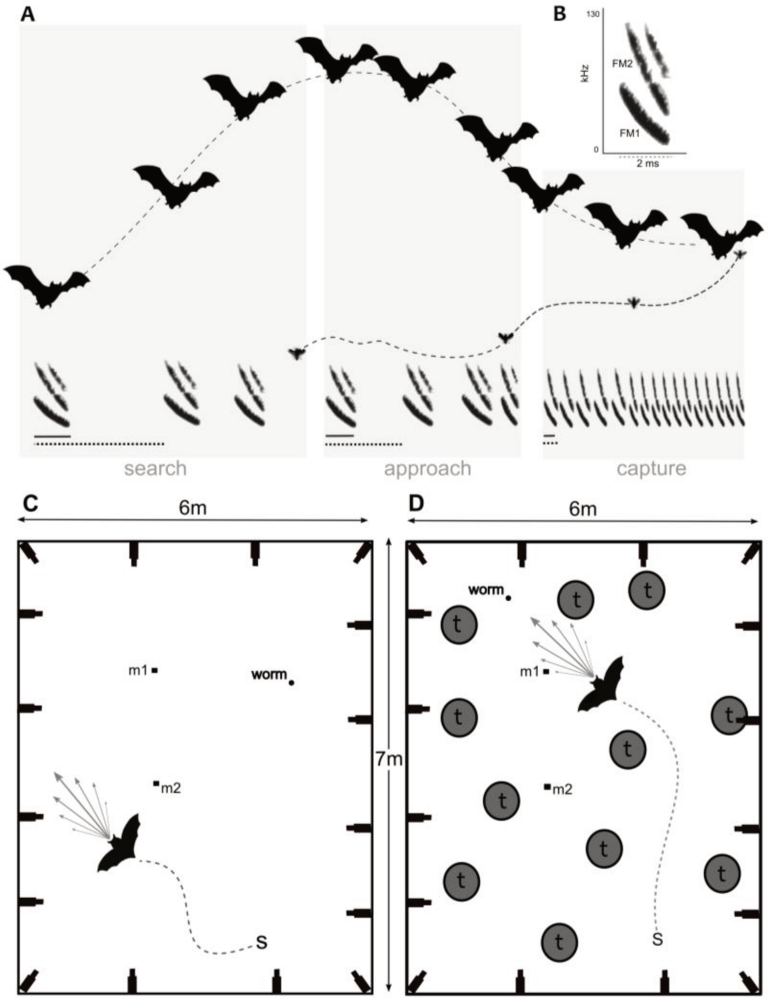
pair, silent behavior was observed for almost 70% of analyzed trial time (Chiu et al., 2008). Bats showed the greatest silent behavior when paired with a conspecific whose signals were naturally similar in spectral characteristics, as measured in baseline recordings, from animals flying alone in an open room. In fact, the more similar the individual bat's echolocation calls were to another bat's signals in baseline recordings, the more likely it was that one bat would go silent when the two were paired (see Figure 4B of Chiu et al., 2008).

Chiu et al.'s studies (2008, 2009) show adaptive sonar behaviors of bats, which serve to minimize jamming avoidance; however, the findings are restricted to situations in which echolocating bats are foraging in open spaces. While big brown bats are known to navigate and forage in open spaces (Simmons, et al., 2001), they have also been observed to successfully capture prey in heavily cluttered environments, such as forests (Simmons, 1973; Simmons et al., 2001; Surlykke et al., 2009).

Here, we extended Chiu et al.'s work by investigating vocal and flight adaptations in paired big brown bats that competed for a single prey item in a cluttered space. Specifically, we investigated whether two bats competing for a single prey item (1) adjusted their sonar call structure to avoid sonar interference from a conspecific and (2) exhibited silent behavior when they foraged and navigated obstacles in the presence of a conspecific. We hypothesized that bats would adjust parameters of their sonar calls in the cluttered environment to negotiate obstacles when flying alone and in pairs, similar to findings reported by Chiu et al. (2009). Additionally, we hypothesized that paired bats would exhibit little or no silent behavior in the presence of obstacles, as each bat must continuously monitor its surroundings. The results of this study shed light on behavioral

and vocal adaptations of echolocating bats as they transition from open space to cluttered environments, and specifically address how bats minimize interference from the calls of conspecifics, while also monitoring the location of obstacles.

**Figure 5. 1. Vocal adaptations during prey pursuit and flight room arrangement.**




(A) Schematic of successive positions of an insectivorous echolocating bat searching for approaching and capturing an insect. Foraging process is subdivided into 3 parts (search, approach, capture) during which the call rate increases (see spectral depictions on bottom), and the duration of each call (solid black line) and pulse-interval (PI, black dots) decrease with decreasing distance of the bat to the insect (dashed lines). (B) Typical *E. fuscus* echolocation call. Example of a short, 2ms long broadcast sweeping down from ~130 to ~25 kHz in two prominent harmonics, FM1 and FM2. (C) Flight room configuration for Empty Room conditions (S-ER, ER). Walls and ceiling of flight room (7 x 6 x 2.5 m, L x W x H) were covered in acoustic foam to minimize reverberation and echoes from walls. Two ultrasonic microphones (m1, m2) were positioned on the floor. Sixteen motion-capture cameras were mounted around the perimeter of the room to capture the bat's flight and recreate a 3D flight path (grey dotted line) as the bat searches for the worm (black dot labeled worm). Bat's broadcast is depicted in grey arrows. (D) Flight room setup during Forest conditions (S-F, F). Black circles represent artificially introduced trees (t). Setup is the same for baseline (depicted here) and experimental conditions. Illustration is not to scale.

*Materials and Methods*

*Animals*

Six wild-caught female big brown bats, *E. fuscus* were individually trained to catch a tethered mealworm during flight. Bats' weights were maintained between 14 and 16 g, and they received mealworms (*Tenebrio molitor*) daily to maintain this weight for the period of training and testing. Bats were housed in individual cages under reversed 12-hour light/dark-cycle in a colony room kept at 24 to 28 deg C at 30 to 50 % relative humidity. The experimental procedures were approved by the University of Maryland Animal Care and Use Committee.

*Behavioral experiments*

This study investigated vocal and flight behavior of bats, alone and in pairs, in an empty room and in an artificial forest. Experiments were run between July and September 2013 in a large flight room (7 x 6 x 2.5 m) equipped with 2 ultrasound microphones placed on the floor, and a sixteen-camera motion tracking system (Figure 1, see below). Only dim long wavelength lighting (Reed plastic filters, > 650 nm red filter) was used during experimental and baseline flights to restrict bats from using visual cues (Hope and Bhatnagar, 1979).

Each of the six bats was first individually trained to catch a tethered mealworm in an empty flight room. Once each bat had reached at least 80% success, baseline experiments began. All six bats took part in two baseline (single bat empty room and single bat forest condition) and two experimental conditions (paired bats empty room and paired bats forest condition). Two experimenters were present for each trial of the

experiment. In paired trials, bats were individually marked with reflective markers to identify and distinguish between them in video recordings. After the tethered insect was set up and the recording equipment was ready for a trial, the experimenter released the single bat or two bats simultaneously into the flight room. Bats were free to fly until one of them caught the mealworm. Once the mealworm was caught, a second experimenter end-triggered the synchronized data acquisition system, which saved 8 seconds of the preceding audio and video recordings. After the audio and video data were saved, a new trial was started.

To replicate the study of Chiu et al. (2008) we ran the first baseline condition (S-ER) with each single bat flying in an empty room (Figure 1C). Subsequently, we conducted a second set of baseline trials, flying individual bats in a "forest," with artificial tree trunks constructed from nets hanging from circular 20 cm diameter rings (S-F, Figure 1D). After each bat completed the baseline conditions in the open room and in the forest, individual bats competed for a tethered insect in the presence of another bat. All possible combinations of bat pairs were tested (15 pairs) in the empty room (P-ER) and forest (P-F) conditions. The bat release points varied throughout both baseline and experimental conditions. For the analysis, we used all trials available with good quality audio and video recordings (S-ER: N = 75, S-F: N = 88). Data for paired condition analysis was also selected according to the quality of audio recordings and reliable 3D flight path reconstruction (P-ER: N = 121, F: N = 110). We analyzed data from 13 of 15 bat pairs, as the signal:noise ratio of the audio data for two bat pairs was not high enough to extract echolocation call parameters.

*Data recording*

For each trial, we recorded audio and video data. Audio data were recorded using two ultrasonic microphones (UltraSound Advice, London, UK), filtered (10-100 kHz), and digitized at a sample rate of 250 kHz (National Instruments). Flight data were recorded using a sixteen-camera infrared (IR) motion tracking system (Vicon Motion Systems Ltd., UK), which was mounted along the perimeter of the room (Fig. 1C/D) and tracked IR reflective markers attached to each bat. The Vicon motion tracking system also acquired data on the position of the floor microphones, the tethered mealworm and the obstacles mimicking the artificial forest in the flight room. Synchronized Vicon cameras ran at 300 frames/second. Data from the tracking system were used to reconstruct 3D bat flight paths.

Audio and video data were analyzed off-line using custom-written MATLAB programs (Mathworks, Natick, MA, USA).

*Data analysis*

For baseline conditions (S-ER, S-F), data analysis required tracking the bat's position in space at points sampled every 3.3 ms, and analyzing the bat's corresponding acoustic emissions. A custom-written MATLAB program was used to track the bat's flight position and manually work through the audio recording of each trial and mark a single bat's start- and end-frequency, as well as start- and end-time of each call emitted in a given trial. These markings allowed us to later analyze six signal parameters of interest: start- and end-frequency, bandwidth, sweep rate, pulse interval (PI) and duration. We analyzed data starting at 3 seconds before the final buzz in each trial, and only used data

points during confirmed bat flight. Buzz calls were excluded from analysis.

Central to the analysis of this data set was assigning each call to the vocalizing bat in both paired conditions (P-ER, P-F). After reconstruction of each bat's flight path, these position data were loaded into a custom-written MATLAB program to combine it with the recorded audio signals from the two floor microphones. Loading the position data into the audio analysis program allowed us to use the time difference of arrival of the signals at each of the microphones and assign calls to one bat or another by referencing to their marked location in the room at the time of call emission. For a more detailed description of this process refer to Chiu et al. (2009). Only time segments with reliable audio recording and 3D-flight path reconstruction were used for analyses, and as noted above, buzz calls were excluded.

All echolocation call measurements (start- and end frequency, bandwidth, sweep rate, PI, and duration) were taken from the FM1 component, for all bats and all conditions. After pre-processing, raw data were analyzed by discriminant function analysis (DFA, SPSS), using the measured raw call variables to assess the similarity of call design between bats (cf. Chiu et al., 2008). Differences of raw call features as the bat transitioned from single to paired and empty to forest were assessed by running full factorial (2-level) ANOVAs with bat as a random factor in JMP. A total of 546 calls were identified as outliers (> 3SDs, 1.79%) and excluded from the analysis. *Adjustments* of call parameters in the single conditions were calculated by taking the difference between each successive call feature for the duration of the trial for each individual bat when it flew alone in S-ER and S-F. Call adjustments for paired conditions were calculated the same way for each individual bat. This was possible, because video position data,

combined with measurement of signal onset times at each microphone, allowed us to correctly assign each call to the vocalizing bat. Differences between *call adjustments* on the level of single/paired and empty room/forest were calculated using full factorial ANOVAs with bat as a random factor in JMP. 369 call adjustment measurements were identified as outliers (> 3SDs, 1.23%) and excluded from the analysis.

*Results*

The present study aimed to identify behavioral and vocal changes in big brown bats as they foraged alone in an open space or a cluttered environment, as well as paired individuals in the same environments, where the two bats competed for a single prey item.

Our results show that bats adapt the temporal and spectral structure of their vocalizations, as well as their flight behavior, when they transition from single to paired flight, and from empty to cluttered environments. Further, we report that bats make a larger adjustment of call parameters when navigating cluttered environments, compared to open spaces (see below).

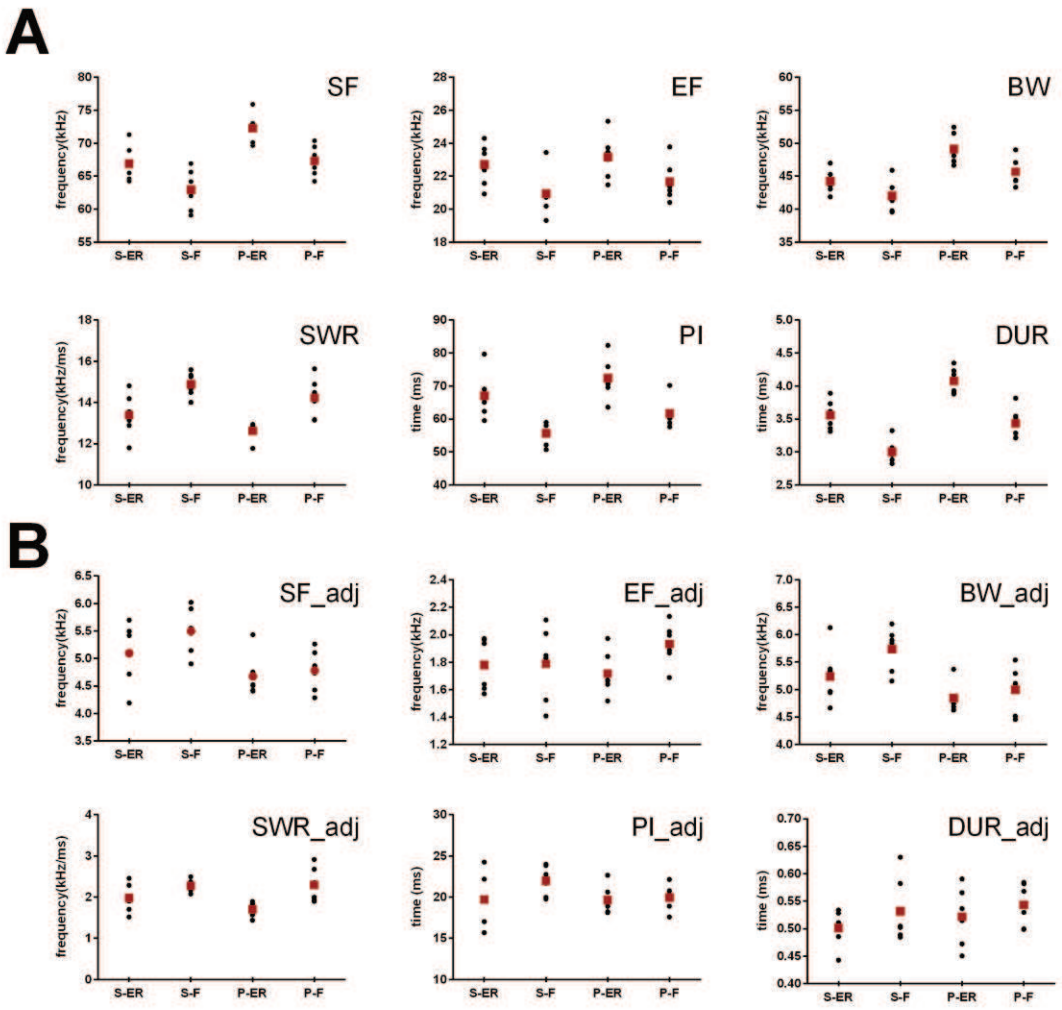
The mean call features (black square) taken across individual bats (grey circles) and all four conditions (x-axis) are plotted in Figure 2A. After Bonferroni correction ($p = 0.0083$), all variables show a main effect of condition (single/paired) and environment (forest/empty). Specifically, as bats transition from flying alone to navigating in pairs, bat calls show an increase in start frequency ($F(1,593) = 178.24$, $p < 0.0001$, single: 64.79 kHz, SE = 0.96, paired: 69.82 kHz, SE = 0.95), end frequency ($F(1,593) = 17.49$, $p <$

0.0001, single: M = 21.80 kHz, SE = 0.45, paired: M = 22.41 kHz, SE = 0.40), bandwidth ($F(1,593)$, = 151.37, $p < 0.0001$, single: M = 42.98 kHz, SE = 0.759, paired: M = 47.42 kHz, SE = 0.78), PI ($F(1,593) = 26.07$, $p < 0.0001$, single: M = 61.22 msec, SE = 2.21, paired: M = 66.98 msec, SE = 2.13), and duration ($F(1,593) = 159.3$, $p < 0.0001$, single: M = 3.26 msec, SE = 0.09, paired: M = 3.75 msec, SE = 0.11), as well as a decrease in sweep rate ($F(1,593)$, $p < 0.0001$, single: M = 14.13 kHz/msec, SE = 0.31, paired M = 13.41 kHz/msec, SE = 0.3). This suggests that the presence of another echolocating bat evokes dynamic changes in sonar vocalizations to navigate the environment successfully. There was also a main effect of environment in all measured call parameters showing changes in spectral parameters as bats transitioned from empty room to forest conditions in start frequency ($F(1,593) = 137.35$, $p < 0.0001$, empty: M = 69.54 kHz, SE = 1.00, forest: M = 65.06 kHz, SE = 1.04), end frequency ($F(1,593) = 121.94$, $p < 0.0001$, empty: M = 22.92 kHz, SE = 0.36 , forest: M = 21.29 kHz, SE = 0.37), bandwidth ($F(1,593) = 61.88$, $p < 0.0001$, empty: M = 46.62 kHz, SE = 0.92, forest: M = 43.78 kHz, SE = 0.79), and sweep rate ($F(1,593) = 113.7$, $p < 0.0001$, empty: M = 12.99 kHz/msec, SE = 0.23 , forest: M = 14.55 kHz/msec, SE = 0.23). Temporal call parameters show significant changes in PI and duration when bats flew in the forest compared to the open room, as indicated by a lower PI during flights in the forest ($F(1,593) = 92.73$, $p < 0.0001$, empty: M = 69.63 msec, SE = 1.83, forest: 58.58 msec, SE = 1.42), and shorter call durations in the forest ($F(1,593) = 233.33$, $p < 0.0001$, empty: M = 3.8 msec, SE = 0.09, forest: M = 3.21 msec, SE = 0.08). Analyses revealed no significant interactions of condition by environment.

The *adjustments* in call parameters that bats made from call to call as bats foraged

in each of the conditions are presented in Figure 2B, plotting the average adjustments made by a given bat for each call parameter (grey circles), along with the mean adjustments across conditions (black square). After Bonferroni correction ($p = 0.0083$), the call adjustments that bats made when tested in single flight compared to paired flight show a main effect of condition (single/paired) only in start frequency ($F(1,593) = 30.92$, $p < 0.0001$, single: $M = 5.32$ kHz, $SE = 0.08$, paired: $M = 4.91$ kHz, $SE = 0.04$) and bandwidth ($F(1,593) = 31.13$, $p < 0.0001$, single: $M = 5.51$ kHz, $SE = 0.08$, paired: $M = 4.92$ kHz, $SE = 0.04$). There was a main effect of environment, such that bats consistently made larger adjustments from one call to the next when flying in the forest, compared to the open room (start frequency: $F(1,593) = 8.34$, $p < 0.0001$, empty: $M = 4.76$ kHz, $SE = 0.06$, forest: $M = 4.99$ kHz, $SE = 0.06$, end frequency: $F(1,593) = 8.55$, $p = 0.0036$, empty: $M = 1.72$ kHz, $SE = 0.02$, forest: $M = 1.89$ kHz, $SE = 0.02$, bandwidth: $F(1,593) = 14.8$, $p = 0.0001$, empty: $M = 4.91$ kHz, $SE = 0.05$, forest: $M = 5.21$ kHz, $SE = 0.06$, sweep rate: $F(1,593) = 72.82$, $p < 0.0001$, empty: $M = 1.77$ kHz/msec, $SE = 0.03$, forest: $M = 2.29$ kHz/msec, $SE = 0.03$, duration: $F(1,593) = 9.0$, $p = 0.0028$, empty: $M = 0.51$ msec, $SE = 0.006$, forest: $M = 0.54$ msec, $SE = 0.005$, PI: $F(1,593) = 7.19$, $p = 0.0075$, empty: $M = 19.7$ msec, $SE = 0.34$, forest: $M = 20.6$ msec, $SE = 0.29$). These results show that bats make greater adjustments in their call structure while navigating cluttered environments, compared with open spaces. Further, sweep rate is the only variable showing a significant interaction of condition by environment ($F(1,593) = 7.72$, $p = 0.0056$): The cluttered environment revealed greater adjustments in the bat's sweep rate when it flew by itself or in a pair.

**Figure 5. 2. Changes in call features and call adjustments across conditions.**




(A) Averages (black square) across bats (grey circles) of the respective raw call feature variable in each condition (S-ER, S-F, P-ER, P-F). (B) Mean value of call-to-call *adjustment* across bats (grey circles) for each condition (S-ER, S-F, P-ER, P-F). Black square plots mean value across bats per condition to illustrate main effects and interactions between main effects (see Results).

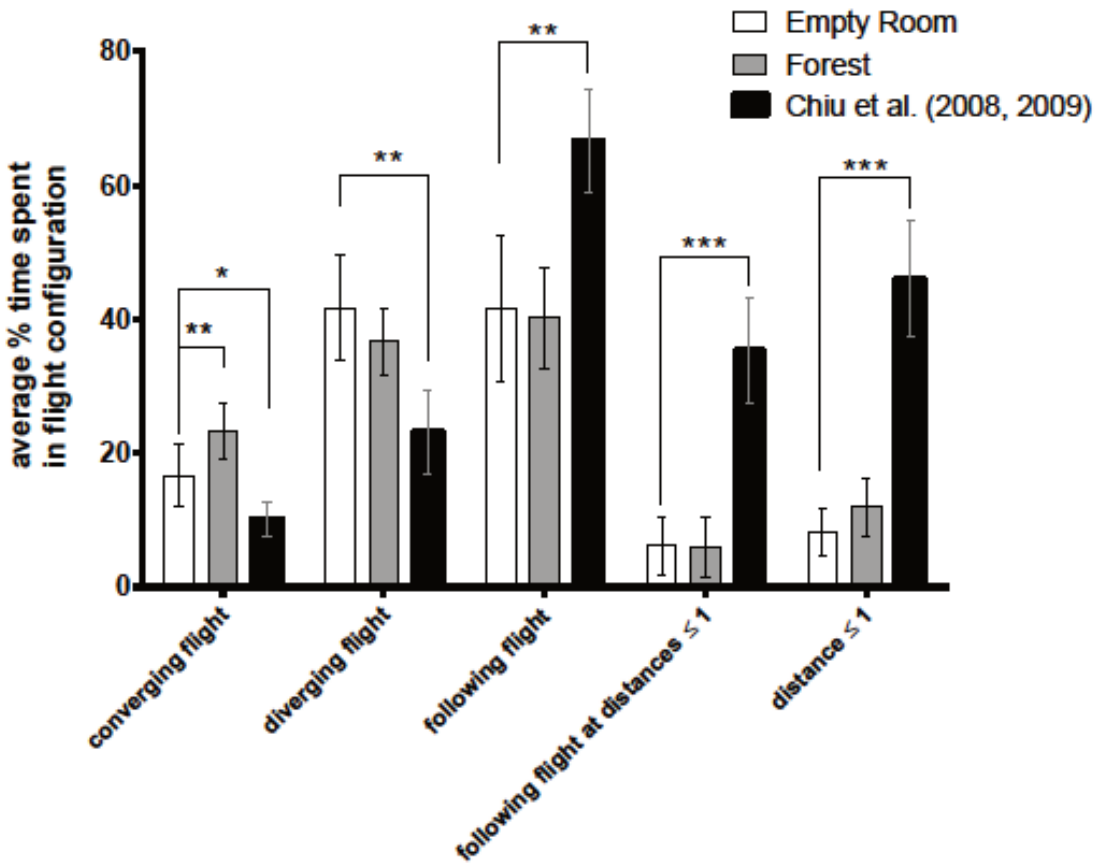
A second mechanism to avoid interference from the acoustic signals of conspecifics, reported by Chiu et al. (2008), is silent behavior in paired bats (Chiu et al., 2008). We hypothesized that paired bats would exhibit silent behavior in the paired empty room condition (P-ER), and they would exhibit reduced, if any, silent behavior in all other conditions (S-ER, S-F, P-F). Our data show an absence of silent behavior in single bat conditions, as well as while paired bats fly in a cluttered environment (S-ER:

M = 0.44%, SD = 0.69%, S-F: M= 0.46%, SD = 0.46%, P-F: M = 0.27%, SD = 0.72%). However, we also did not find evidence for silent behavior in the empty room paired bat condition (P-ER: M = 0.73%, SD = 0.99%).

Given Chiu et al.'s (2008) report of silent behavior of paired bats in an open room (on average, 40% of analyzed trial time when inter-bat separations were ≤ 1 m), the absence of silent behavior in this study motivated a closer investigation of the flight behavior of paired bats in the empty and cluttered environments. Chiu et al. (2008) reported that silent behavior was most prevalent when the inter-bat distance was ≤ 1 m, and additionally, when the two bats were in a following-flight configuration (Chiu et al., 2008). Two-tailed paired samples t-tests revealed that bats in the present study did not exhibit significantly different flight behaviors in open or cluttered environments, with the exception of converging flight (Figure 3). Figure 3 illustrates flight configuration data and, importantly, shows that the bats used in this study spent only 8-10% of their time flying at distances of ≤ 1m. Even less time was spent at 1 m or closer distance while in following flight.

Although paired bats spent similar amounts of time in diverging flight (P-ER: M = 41.14%, SD = 10.82%, P-F: M = 36.59%, SD = 4.81%) and following flight (P-ER: M = 43.79%, SD = 13.61%, P-F: M = 38.88%, SD = 8.66%) in P-ER and P-F conditions, the amount of time spent converging in the cluttered environment (M = 24.72%, SD = 6.48%) was significantly higher than in the empty room (M = 15.06%, SD = 6.24%, $t(12) = -4.005$, $p = 0.002$). Further, there is no significant difference of inter-bat distance in the two environments, and no significant difference in the amount of time two bats spent following each other at distances of ≤ 1m.

**Figure 5. 3. Flight configurations during P-ER/P-F, and Chiu et al. (2008)**




Bar graphs plot the percent of time (y-axis) all bats spent in a different flight configuration (x-axis) of P-ER (white) and P-F (grey) conditions, as well as during Chiu et al. (2008, 2009; black). Bats spent little time at distances ≤ 1m or in following flight configurations. Flight configurations differ significantly between P-ER and Chiu et al. (2008, 2009). Bats in the current study spent less time following one another or at close proximity, an indication of increased call adjustment and silent behavior.

## *Discussion*

Echolocating big brown bats forage in open spaces, as well as in heavily cluttered vegetation (Simmons, 1973; Simmons et al., 2001; Surlykke et al., 2009). The presence of physical obstacles poses challenges to the echolocating bat, as it has to monitor its surroundings continuously to avoid collisions. Recent work has shown that single bats adjust temporal characteristics of their calls in cluttered environments, compared to open spaces (Falk et al., 2014). Falk et al. (2014) report a decrease in call duration and flight

speed, along with an increase in call rate, when bats fly in an artificial forest compared with an empty room. Our results are consistent with these findings (see Table 1) and further indicate that bats additionally modulate all parameters that we measure in this study when they fly in a cluttered environment (Figure 2).

Table 1. 1 Mean flight velocity and sonar call rate in baseline trials

| | **Flight Velocity (m/s)** | | | | **Mean Call rate per trial** | | | |
|---|---|---|---|---|---|---|---|---|
| **Bat** | **S-ER** | **S-F** | **P-ER** | **P-F** | **S-ER** | **S-F** | **P-ER** | **P-F** |
| **G82** | 2.72 | 2.77 | 3.34 | 2.96 | 27.42 | 41.51 | 29.01 | 26.61 |
| **O33** | 2.81 | 2.62 | 3.12 | 2.91 | 26.44 | 38.59 | 27.32 | 30.93 |
| **O92** | 2.50 | 2.34 | 2.88 | 2.69 | 30.51 | 41.88 | 24.36 | 27.46 |
| **R14** | 2.93 | 2.54 | 3.30 | 2.92 | 27.42 | 42.39 | 26.52 | 29.64 |
| **R20** | 2.62 | 2.53 | 2.94 | 2.70 | 28.79 | 48.70 | 31.02 | 27.43 |
| **R24** | 3.05 | 2.63 | 2.52 | 2.38 | 24.29 | 42.94 | 33.18 | 28.79 |
| **mean** | 2.77 | 2.57 | 3.02 | 2.76 | 27.48 | 42.67 | 28.57 | 28.48 |
| **SD** | 0.20 | 0.14 | 0.31 | 0.22 | 2.11 | 3.32 | 3.19 | 1.62 |
| **significance** | | | **F-** | **P –** | | | **F-** | **P -** |
| | **velocity** | **DFDen** | **ratio** | **value** | **call rate** | **DFDen** | **ratio** | **value** |
| **test** | **S/P** | 15 | 7.46 | 0.016 | **S/P** | 15 | 34.16 | <.0001 |
| | **ER/F** | 15 | 8.39 | 0.011 | **ER/F** | 15 | 45.34 | <.0001 |
| | **Interaction** | 15 | 0.13 | 0.728 | **Interaction** | 15 | 46.48 | <.0001 |

Summary of baseline flight velocity and call rate. Each cell represents the average flight velocity per bat or call rate of that bat across all trials analyzed in S-ER, S-F, P-ER and P-F, respectively. Means and standard deviations are added, along with significance between each condition (2-way ANOVA, bat added as random factor, JMP).

Foraging in their natural habitat, however, not only poses the challenge of negotiating physical objects, but also that of acoustic interference from nearby foraging conspecifics. Previously, it was shown that echolocating bats make use of two different strategies to overcome the challenge of acoustic interference from other bat signals.

Chiu et al. (2009) report that one strategy to avoid acoustic interference is dynamic vocal adjustment in the spectral features of calls, namely start-, end frequency, and bandwidth of the FM sweep. Furthermore, call adjustments were largest when bats flew at inter-bat separations of $\leq 1$ m.

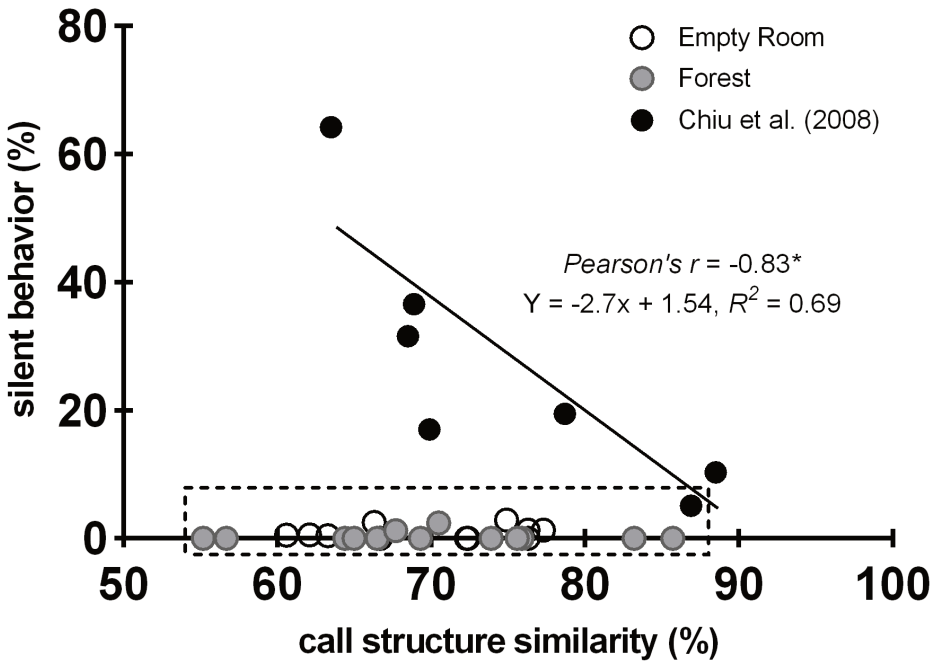
In the present study, we found reliable vocal changes in paired bats, similar to those reported by Chiu et al. (2009). Specifically, our data show that when bats adapt from flying alone to being paired with a conspecific, they show significant changes in call features. In the present study, there were main effects of condition (single/paired) and environment (empty/forest) on bat echolocation call design, suggesting that adaptive call behavior is influenced by the presence of another conspecific *and* the surrounding environment. In other words, both the potential acoustic interference of another bat and physical obstacles drove changes in bat call design.

Given changes in bat call design across conditions, we considered how these adjustments differ between single or paired conditions, and in empty or cluttered environments. Our results indicate that bats make larger adjustments of all call parameters when they are navigating in a cluttered environment, as compared to an open space. Further, bats make significant adjustments in start frequency and bandwidth when they fly with a conspecific compared with flying alone. Collectively, these findings suggest that adjustments in call parameters from one vocalization to the next allows the

bat to better distinguish its own call and echo from the other bat's signal.

Chiu et al. reported that call adjustments and silent behavior were most prevalent at inter-bat distances of ≤ 1m (Chiu et al., 2009, 2008). Surprisingly, in the present study, silent behavior was not observed in the empty room paired bat condition (P-ER), which prompted an investigation of flight behavior in both paired conditions (P-ER, P-F). Comparing the amount of time that bats in the present study spent separated by ≤ 1m (M = 9.1%, SD = 3.9%) with that reported by Chiu et al. (2008, 2009; M = 45.89%, SD = 8.6%), it becomes evident that the bats in this study spent significantly less time in close proximity ($t(20) = -14.038$, $p = 0.0001$, Figure 3, black bars: Chiu et al., 2008), and also significantly less time engaged in following flight at this separation ($t(18) = 20.539$, $p = 0.0001$, Figure 3). In fact, Figure 3 shows that following flight occurred significantly more often in Chiu et al.'s data set compared to the current study ($t(2) = -3.187$, $p = 0.005$), and also that bats in the current study showed significantly more converging ($t(18.588) = 2.140$, $p = 0.046$) and diverging flight ($t(20) = 3.585$, $p = 0.002$) than reported by Chiu et al (2008, 2009). It is noteworthy that Chiu et al. (2008, 2009) did not report increased call adjustments when bats engaged in converging and diverging flight paths. In contrast to the bats in Chiu et al.'s study, which spent more than half of their time engaged in following flight at close inter-bat separations (≤ 1m), bats in the present study were engaged predominantly in converging and diverging flights, an indication of uncoordinated flight patterns between the individuals in the pair. The differing flight configurations of bats in the two studies could possibly account for reported differences in vocal behavior.

**Figure 5. 4. Comparison of silent behavior of Chiu et al. (2008) and current data.**




Scatter plot shows silent behavior (% silence, y-axis) by similarity of baseline call structure (%, x-axis). Data illustrate distinct differences between distributions of the silent behavior in the current P-ER (white circles) and P-F (grey circles) conditions, compared to silent behavior reported in Chiu et al. (2008, black circles). Dotted rectangle includes all female-female bat pairs run in both studies.

A second strategy employed by big brown bats to avoid acoustic interference with conspecifics is silent behavior (Chiu et al., 2008). Bat silent behavior is correlated with baseline call design similarity of two bats, such that two bats with very similar baseline call design showed more silent behavior than paired bats with less similar baseline call design (Chiu et al. 2008). In the present study we hypothesized that bats would show silent behavior in the empty room condition when flying in pairs, similar to reports by Chiu et al. (2008). To our surprise, however, the bats in the present study exhibited very little silent behavior in the empty room (P-ER), or the forest (P-F) condition. In fact, the small amount of silent behavior that was observed in the current study differs significantly from that reported by Chiu et al. ($t(6.017), = -3.7, p = 0.01$). Figure 4 plots the percentage silent behavior as a function of baseline call similarity between paired bats

(correctly classified by DFA) from Chiu et al. 2008. Along with the data from Chiu et al. (black circles) we plotted the data points from the current study for silent behavior in paired empty (P-ER, white circles) and paired forest (P-F, grey circles) conditions, with their respective correctly classified baseline DFA scores. This plot reveals that the data sets collected in the two studies are distinctly different.

The surprising finding that paired bats in the open room showed little silent behavior in this study led us to investigate the factors that could possibly contribute to differences from the findings reported by Chiu et al. (2008). Just as the distinct patterns in flight behavior observed in the two studies could account for differences in call adjustments, different flight configurations could possibly explain the absence of silence.

In addition to differences in flight configurations of paired bats in the two studies, we note differences in the gender composition of subjects in the present study, compared to those in Chiu et al. (2008, 2009). Specifically, animals in the present study were exclusively female, creating 15 female-female pairs, 13 of which were used for analysis. In contrast, Chiu et al. (2008) used five male and three female bats, and tested three male-male, three male-female and only one female-female pair. Interestingly, the one female-female pair used in Chiu et al. showed only about 5% silent behavior of analyzed trial time in the open room, compared to up to 70% in one male-male pair. For better visualization, we have enclosed all female-female pairs from the current study and the one female-female pair of Chiu et al.'s data in a dashed black rectangle in Figure 4. While all female bats in the current study employed circular flight behavior patterns, Chiu et al. (2008) report a predominance of following flight behavior between paired bats. In fact, Chiu et al.'s single female-female pair showed the least total amount of

following flight behavior (55.82%) and even less following flight behavior at distances of ≤ 1m (27.11%), compared to all other pairs tested in her study averaging 68.40% (following) and 36.72% (following ≤ 1m), respectively. Additionally, we analyzed data from Chiu et al.'s previous studies (2008, 2009) and confirm that in male-female pairs, the male bat consistently exhibited more silent behavior than the female bat (Table 2).

Table 1. 2 Silent behavior in male and female bats, Chiu et al., 2008

| bat pair | | gender | | silence (ms) | | total % |
|---|---|---|---|---|---|---|
| bat 1 | bat 2 | bat 1 | bat 2 | bat1 | bat2 | silence/pair |
| g32 | g48 | f | f | 29.77% | 70.23% | 5.09% |
| p17 | hp54 | f | m | 17.15% | 82.85% | 17.01% |
| p17 | y31 | f | m | 3.42% | 96.58% | 19.47% |
| g48 | g78 | f | m | 26.91% | 70.93% | 31.56% |
| hp54 | y31 | m | m | 60.24% | 39.76% | 10.31% |
| g67 | g78 | m | m | 17.55% | 69.37% | 64.17% |
| g41 | g78 | m | m | 18.35% | 79.49% | 36.57% |

Summary of silent behavior separated by gender for each bat used in Chiu et al., 2008. Each cell represents the percent silent behavior that a given bat exhibited throughout the length of all analyzed trial time. Female bats' silent behavior is shaded grey. Last column shows total silent behavior for a given bat pair. Whenever female bats were paired with male bats, males produced more silent behavior than females. Female-female pair showed least silent behavior.

Taken together, the data reported here suggest a possible gender difference in the strategies used by bats to avoid acoustic interference while foraging with conspecifics. Results from Chiu et al. (2008, 2009) indicate that male bats engage in chasing-like behavior, following one another at close inter-bat distances in flight, ceasing echolocation and thereby possibly eavesdropping on the other bat. By contrast, the data from the present study, along with a subset of data reported by Chiu et al., show that female bats, when paired together, engage in more circular flight paths at greater inter-bat distances and avoid being close to one another, thereby reducing acoustic interference from the

conspecific, and hence show less silent behavior, as well as smaller adjustments in their echolocation call design. It is important to note, however, that these studies were conducted at different time points and include bats from different colonies.

In summary, we report strategies that bats employ to avoid acoustic interference, not just from nearby echolocating conspecifics, but also while navigating in cluttered spaces. The intriguing differences between the data collected from female bats in this study and the male bats in Chiu et al.'s studies raise compelling questions about gender and social hierarchy influences on flight and echolocation behavior in the big brown bat. These questions provide fertile ground for future research.

# Chapter 6

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# The mystery of silent behavior and its function

Completed Experiment Synopsis: Previous work has established that big brown bats foraging among conspecifics will sometimes change characteristics of their vocalizations or cease to echolocate (Chiu et a., 2008, 2009). Warnecke et al., (2015; Chapter 5) extended that work to test how cluttered environments affect these behaviors, but were unable to replicate the silent behavior reported by Chiu et al., (2008). In-depth analysis revealed that bats in Chiu et al., (2008) spent significantly more time at close distances (< 1 m) and in following flight than bats in Warnecke et al. (2015), and all but two bats in Chiu et al. (2008) were male, while all bats in Warnecke et al. (2015) were female. Apart from potentially being impacted by gender or flight configuration, silent behavior may also depend on other factors such as competition during foraging, social hierarchies, or learned techniques. In a large-scale study, we let nine bats of different genders navigate a variety of conditions both by themselves and in pairs.

To investigate on the function of silent behavior, we here focus on the condition that tests single and paired bats in an open space while competing for a single prey item (see Chiu et al., 2008).

**Title: Adaptive echolocation and flight behaviors of paired big brown bats during foraging**

Michaela Warnecke, and Cynthia F. Moss

*Introduction*

Echolocating bats move through their environment with ease despite facing a complex acoustic soundscape that comprises their own calls and echoes, other conspecifics' calls and echoes, as well as any natural sounds that arise from the environment (Moss and Surlykke, 2001, 2010; Schnitzler et al., 2001; Simmons et al., 2001; Surlykke and Kalko, 2008). The bat's ability to forage and navigate successfully depends not only on the signal-to-noise ratio of echoes and background sounds, but also on the bat's ability to perceptually separate different sound sources and correctly interpret acoustic patterns to adjust flight behavior accordingly.

Recent work has shown that bats decrease their flight speed, as well as call duration and increase call rate when they navigate cluttered environments (Falk et al., 2014). While surrounding objects comprise much of the physical clutter in a bat's soundscape, acoustic interference can arise from calls and echoes of nearby conspecifics. Over the past decade, several studies have shown that bats dynamically modulate spectral and temporal components of their echolocation calls to minimize interference (Chiu et al., 2008; Chiu et al., 2009), and that call design is influenced by both environment *and* the presence of other conspecifics (Warnecke et al., 2015). More specifically, Chiu et al. (2008) showed that echolocating bats will sometimes *go silent*, that is cease to echolocate for at least 200 ms, when they are competing for a single prey item in an open flight

space. The authors reported that such "silent behavior" occurred mostly when one bat was following another at a close distance, and was more likely to be observed if the two bats had similar call structures (Chiu et al., 2008). To learn more about the function and mechanisms that drive silent behavior, Warnecke et al. (2015) extended Chiu et al.'s work by repeating the same experimental setup with a new set of bats, and including a cluttered environment condition. They expected and confirmed little to no silent behavior in the cluttered environment, but were surprised to find that silent behavior was also absent in the open flight room trials. After more in-depth analyses of the data set, Warnecke and colleagues hypothesized that silent behavior may be closely related to flight configurations (following flight vs. converging/diverging flight patterns) and/or gender (Warnecke et al., 2015).

Our previous work was unable to replicate Chiu et al.'s reports of silent behavior in the open room, possibly because all experimental subjects were female (Warnecke et al., 2015). Thus, in the present study we used both male and female bats to investigate how the flight and echolocation patterns of the echolocating big brown bat change as a function of environment, and foraging tasks. Specifically, we aimed to improve our understanding of "silent behavior", its driving mechanism(s) and function. In the present study we let single and paired bats navigate either an empty flight room, or one that had "artificial trees" scattered throughout. Further, each of these environmental conditions was crossed with either having no prey, a single prey item or multiple prey items present. These combinations of experimental conditions would allow us to test (1) whether silent behavior is related to gender, (2) whether silent behavior is dependent on the mere presence of another bat, competition during foraging, or general presence of food, and (3)

whether silent behavior is evident in cluttered environments. We hypothesized that silent behavior would be most prevalent in male-male bat pairs in the empty room – single prey condition, an assumption based on results of the previous two studies investigating silent behavior.

## *Materials and Methods*

### *Animals*

In this study, nine big brown bats (five female), *E. fuscus,* were wild-caught and served as experimental subjects. Of these nine bats, seven had been caught just before the beginning of the experiment (August 2016) and were naïve to the experiment and environment. The remaining two bats, B70 (male) and Y59 (female) had been caught previously (August 2015 and April 2016, respectively), and had thus had exposure to the experimental rooms and different training procedures. The bats' weights were maintained between 14 and 18 g, and they received mealworms (*Tenebrio molitor*) daily, either during the experiment, or after data collection (see below) to maintain this weight for the period of training and testing. All animals were kept in one group cage under a reversed 12-hour light/dark-cycle in a colony room kept at 24 to 28 deg C at 30 to 50 % relative humidity. The experimental procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

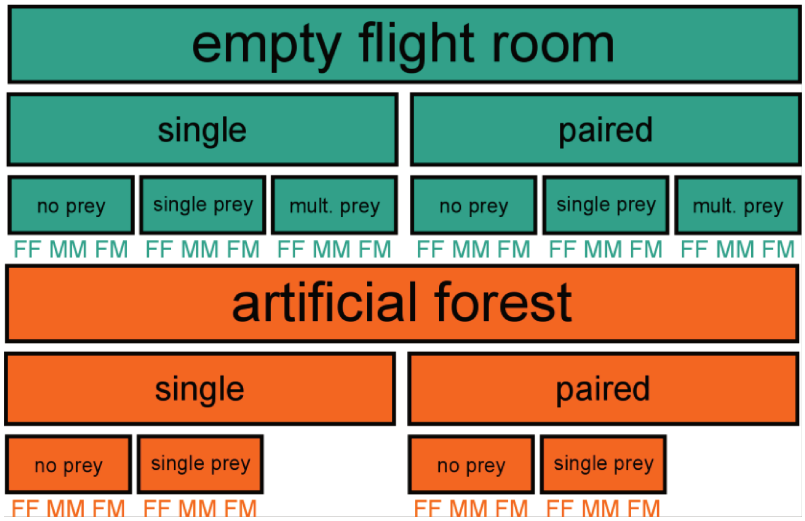
### *Behavioral Experimental Conditions and Setup*

This study investigated the vocal and flight behavior of bats, alone and in pairs, in an empty room and in an artificial forest, while there was different food availability. All

experimental conditions were run between August 2016 and October 2016 in a large flight room (6 x 7 x 2.5 m) that was equipped with 20 ultrasonic microphones and an infrared motion-tracking system (see *Behavioral Data Collection and Analysis*).

Figure 1 illustrates the conditions of this experiment. Bats flew in two environmental conditions (empty flight room, artificial forest), each of which was crossed with one of three conditions of prey availability (no prey, single prey, multiple prey). For each of the combinations of these environmental and prey conditions, bats navigated the space either by themselves (single) or in pairs (paired). Pairs consisted of male-male, female-female or male-female gender combinations. All combinations of recently wild-caught bat pairs were flown in all conditions, but the condition presenting bats with a single prey item (in both empty and forest environments) additionally contained bat pairs that included the two previously-caught bats which had been living in the laboratory colony already (B70, Y59).

**Figure 6. 1. Illustration of conditions of the experiment.**

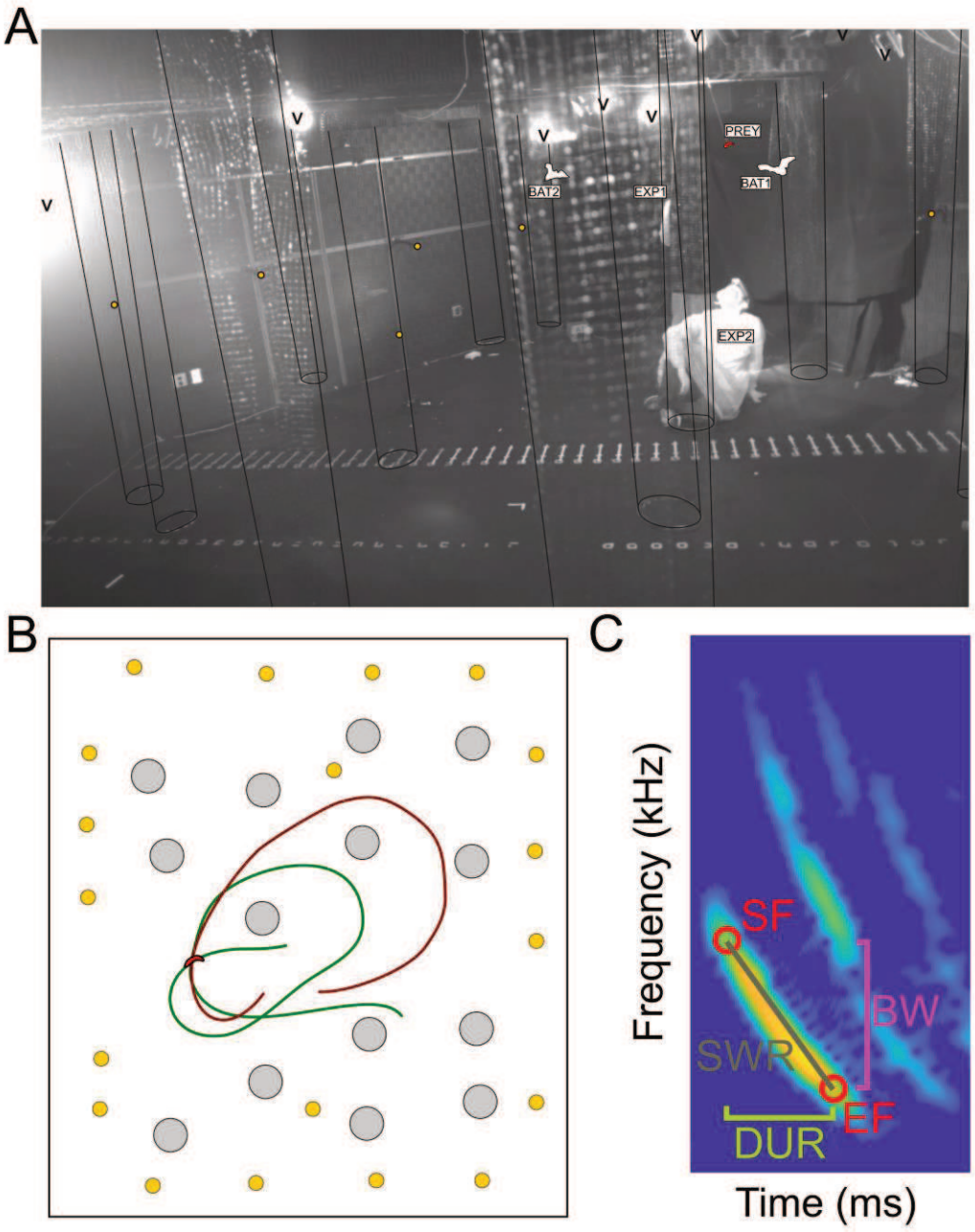



Each panel represents the environment, pairing, prey availability and gender combinations that were part of the current experiment. Single prey conditions had trials of bats that were wild-caught, and bats that had been living in the laboratory for at least 4 months.

Prior to each experiment, an individual bat was removed from the group cage and fit with a custom-built head marker (see Warnecke et al., 2016), attached using water-soluble glue (Grimas Mastix Water Soluble, Heemstede, Holland). The head marker was had reflective spheres glued to it and was positioned between the bat's ears to track the animal's position within the room. In paired trials, bats were individually marked with reflective markers to identify and distinguish between them in infrared (IR) motion-tracking recordings. The experiment started when the marker was securely attached to the bat's head and all recording systems were ready to collect data. Bats were released in a random location within the room and allowed to fly freely until at least ten trials had been collected. After data collection, the head marker was carefully removed, and the animal was returned to a temporary cage. After data from all bats had been collected for the day, all animals were returned to their original colony cage.

For the duration of the experiments on any given day, at least two experimenters were present. One experimenter was responsible for triggering the data acquisition system at the end of a trial and take notes (Figure 2A, "EXP1"), while the remaining experimenter(s) collected the bat(s) at the end of a session, or nudged it (them) to return to flight if it (they) had landed in-between trials (Fig. 2A, "EXP2"). Experimenters were otherwise out of the field of view and flight space of the bat(s).

**Figure 6. 2. Experimental Setup and Call Feature Measurements.**




(A) Snapshot of Miro IR video camera frame during a single prey (red) artificial forest (black outlines around deer-netting) trial. Two bats (white, "BAT1", "BAT2") compete for the prey. Yellow circles indicate wall and floor microphones, "V" indicates Vicon motion-tracking camera. At the time of the experiment, there was no light. Bright white circles beyond "V"s are IR LEDs picked up by the Miro camera. (B) Top-down illustration of data from the same trial in (A). Flight tracks of Bat1 (red) and Bat2 (green) are indicated along with prey location (red), tree locations (grey), and microphone locations (yellow). Trials in empty flight rooms had the same setup, but no trees. (C) Spectrogram of a typical echolocation call with measured call features indicated: Start and End Frequency (red), Duration (green), Bandwidth (pink), and Sweep-Rate (grey).

To guarantee that none of the newly-acquired bats associated the flight room with food as a reward or with food competition, all no-prey conditions were collected prior to any of the conditions involving prey. As such, on the first day after the seven newly-caught bats had been brought to the laboratory, each bat's flight and echolocation behavior was recorded as it flew by itself within the empty flight room. Bats were allowed to fly freely until at least ten trials had been collected. In the no-prey conditions, a trial consisted of at least five seconds of continuous flight, after which an experimenter end-triggered the synchronized data acquisition system. The system saved the preceding 5 seconds of the audio, motion-tracking and video recordings. After the data were saved off-line, a new trial started. When data for all bats had been collected in the empty flight room by themselves, single bats flew without prey in the forest environment. The forest environment was created by hanging 14 "trees" from pre-allocated spaces on the ceiling. Each "tree" was custom-built using two metal rings that had a 25 cm diameter and were connected using 1.7 m of deer-netting wrapped along the circumference of each ring (see Fig. 2A,B). This guaranteed that the bats would detect the tree cylinders as objects to navigate around, while at the same time allowing for the ability to use motion-tracking and video recordings, since the holes in deer-netting rendered it see-through (see Fig. 2A). When all single bat trials without prey had been collected, we paired bats and allowed them to fly in the empty flight room without prey. Once all paired empty room trials had been collected, the same procedure was repeated for the forest environment.

Only after all trials in the no-prey conditions had been collected, the training to capture prey started. Each bat was individually trained to catch a tethered mealworm in an otherwise empty flight room. Once a bat had reached at least 90% success,

experiments for the single-prey condition in the empty flight room began. Each bat was individually released and then allowed to fly freely until it caught the mealworm off the tether, which signaled the end of a trial. At the moment of capture, an experimenter end-triggered the synchronized data acquisition system, which saved the preceding 5 seconds of the audio, motion-tracking and video recordings. After the data had been saved, a new trial started. During the single, but not paired bat conditions in empty environments, prey availability was randomly changed from single to multiple prey. Additionally, prey location was randomly varied from trial to trial to one of three pre-allocated spaces for that day. Once at least ten trials had been collected per single bat in the empty room condition, paired bat data collection for single prey in the empty flight room began. Both bats were released simultaneously into the flight room and allowed to fly freely until one of them caught the mealworm. Once the mealworm was caught, an experimenter end-triggered the synchronized data acquisition system, which saved the preceding 5 seconds of the audio and video recordings. After the audio and video data were saved, a new trial started. When data for all pairs of bats in the empty room conditions for single prey were collected, the same experimental procedure was repeated for the single and paired bats in the forest condition. Throughout all single prey trials, bats were not fed outside of the flight room, meaning that the only food they received was what they caught while in competition with another bat.

To ensure that bats continued to have the drive to compete with one another, the multiple prey condition started only after all single prey trials had been collected. Single bat trials for multiple prey conditions in the empty flight room had already been collected (see above). Unfortunately, we were unable to collect data for paired bats foraging among

multiple prey in the forest condition, as the late time of year had started to induce hibernation behavior in the bats. This caused the animals to be increasingly unwilling to participate in the experiments, as evident by frequent landings on the wall, and difficulty to get bats to fly at all. Thus, we do not have data for the paired bat – multiple prey – artificial forest environment condition.

For all paired bat conditions at least 20 trials were collected. Across all conditions, we analyzed 410 single bat trials and 1,607 paired bat trials. Audio analysis for paired bat trials is time intensive (see below) and has thus been restricted to the condition that was most likely to show silent behavior (paired bat – empty flight room – single prey), a total of 482 trials.

*Behavioral Data Collection and Analysis*

Experiments were run between August and October 2016 in a large flight room (6 x 7 x 2.5 m) equipped with 20 ultrasound microphones, 18 of which were mounted along the perimeter of the walls at a height of about 1.2 m (D500X external microphone, Pettersson Elektronik Uppsala, Sweden), and 2 of which were mounted 35 cm above the floor in the middle of the room (UltraSound Advice, London, UK). All audio data were sampled at 250 kHz (NI PXI board 6143), then band-passed between 10 and 100 kHz (USBPBP-S2, Alligator Technologies CA, USA), and stored for off-line analysis. All bat flight trajectory data, as well as the locations of the prey items, microphones and "trees" that composed the artificial forest environment were collected using a high-speed sixteen-camera infrared (IR) motion tracking system (Nexus, Vicon, Vicon Motion Systems Ltd., UK), which was calibrated before each experimental session. Additionally, we mounted

two IR video cameras on adjacent corners of the flight room to acquire video data of the flights and prey capture moments (Miro Phantom Series, Vision Research, Wayne, NJ, USA; Fig. 2A). For all data collection, only dim long wavelength lighting was used to restrict bats from using visual cues (Hope and Bhatnagar, 1979). Synchronized Vicon and Miro data acquisition ran at 100 frames/second. Both audio and motion-tracking data were stored, processed and analyzed off-line using custom-written MATLAB programs (Mathworks, Natick, MA, USA).

For single bat trials, we extracted the bat location at any point in 3D space, reconstructed the 3D flight path of the bat, and, if appropriate, also the locations of the prey, microphones and "trees". Heat maps of flight paths (Figure 3) were calculated from raw flight data and are normalized across all conditions. To analyze audio data of single bat trials, we first applied threshold detection to find all calls in each 5 s duration audio file across all 20 channels. We then calculated the time-differences-of-arrival (TDOA) between all microphones and compared times of call detections across all channels to ensure that all calls were detected. This process was necessary as the bat's call amplitudes increase and decrease with its distance from each microphone, often causing some calls to be buried in background noise. By comparing detection times across all possible channels, we were able to solve that problem. To restrict the automatic detection from picking up echoes, we set the minimum time between two calls to 8 ms. This meant that at buzzing stages, when the bat was about to capture prey, calls at smaller intervals were not detected. However, at 8 ms intervals, we had enough reliable information to predict that the bat was buzzing, rather than navigating the space actively. Since buzz

calls were generally excluded from audio analyses, this limitation did not present a problem for our audio data analysis. After extracting all calls of single bat trials, we used a custom-written MATLAB script to extract call features of each call (Fig. 2C). Overall, 25,311 calls were processed for single bat trials across all conditions. After the automatic call feature marking, we manually went through every call and checked for correct start and end time/frequency assignment. Overall, 13% of calls were manually edited for their markings, indicating that 87% of single bat calls were marked correctly in the automatic assignment. All subsequent data analyses for single bats excluded buzz calls (duration < 1 ms, PI < 10 ms) and social calls (sweep rate < 4 ms/kHz).

For paired bat trials, we extracted locations of both bats at any point in 3D space and reconstructed the 3D flight paths of each bat. Additionally, if appropriate, we also re-created the locations of the prey, microphones and "trees". Heat maps of flight paths (Figure 7) were calculated from raw flight data and are normalized across all conditions. Subsequently, we used custom-written MATLAB code to automatically detect calls on two out of 20 channels (see Fig. S2, S3; orange). One of the two channels was always audio data from a microphone located on the floor, because the difference in elevation of a "floor microphone" and a "wall microphone" helped disambiguate call assignment in situations that bats were at close inter-bat distances. Subsequently, we manually inspected each assigned call start time and corrected the time point, if necessary. This guaranteed that no echoes were detected and that reliable start time marking was set, rather than peak detection. Such accurate marking was necessary to calculate which bat made which call. Subsequently, we used each bat's location in combination with the location of all microphones to calculate time-difference-of-arrival for each marked call

between the two channels. If call marking between channel 1 and 2 matched within 2 ms, meaning the bats were at least 79 cm apart, we assigned the call to the corresponding bat. If the call marking did not match, the call was marked as "unsure". We then used an additional MATLAB script to manually check each assigned call and assign "unsure" calls based on time of arrival of the same call on any six of the remaining 18 channels.

Silent behavior is defined as a period of time in which at least one bat does not emit a sound for a minimum of 200 ms (Chiu et al., 2008). Thus, by calculating pulse intervals (PIs), the time from the onset of one call to the next, we can extract points in time at which one or both bats had PIs $\geq$ 200 ms. Once silence was detected, we used custom-written MATLAB code to exclude silence periods that occurred after the end of a buzz, because bats commonly make longer PIs at that time point to chew their prey, rather than actively produce a silent period.

Following these processing steps, unless noted otherwise, the single bat audio data were analyzed using a Mixed Model in JMP that had bat added as a random factor, and variables of interest as fixed factors (environment, prey availability, flight configurations). When appropriate, we used a Tukey's HSD test for post-hoc testing. To calculate call similarity and classify environment or prey availability, we used a discriminant function analysis (DFA, JMP). We used a quadratic DFA to classify environment/prey availability in single trials, and linear DFA to asses call similarity. We chose the linear DFA for call similarity because we aimed to compare out data to that of Chiu et al. (2008), who used a linear DFA in their analyses. Call similarity was calculated by entering the baseline call feature data for a given bat pair into the linear DFA model.

*Results*

We collected this data set to investigate changes in the echolocation and flight behavior as single and paired bats navigated different environments with different tasks. For the purpose of this thesis, we chose two foci of the data: (1) we analyzed the flight and echolocation behavior for single bat trials across different environmental and prey availability conditions, and (2) we analyzed flight behavior for paired bat trials across all possible conditions, but focused the echolocation analysis on a subset of trials in which bat pairs had to compete for a single worm in an empty flight room, as we aimed to replicate Chiu et al. (2008)'s finding of silent behavior, which was found under similar conditions.

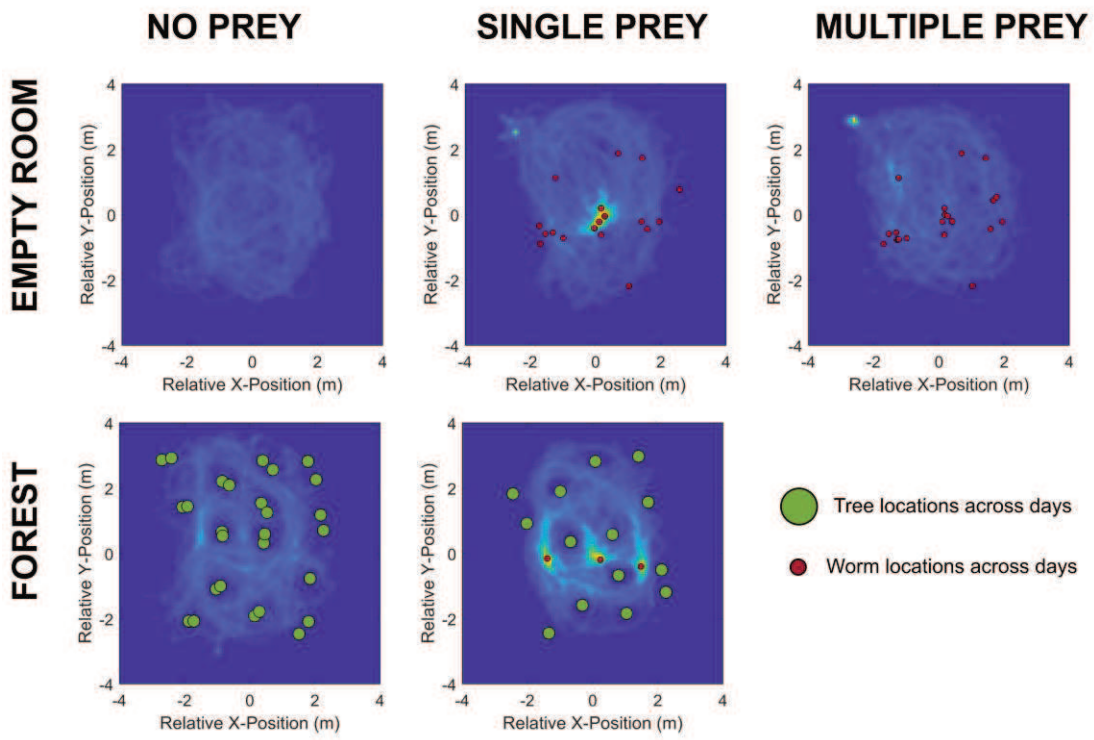
*Single Bat Data*

In baseline trials, individual echolocating bats navigated either an empty flight room or a flight room that had an "artificial forest" set up. Additionally, each of these environments was crossed with different prey availabilities: no prey, a single prey item, or multiple prey items. Due to time limitations, we were unable to collected data in the forest – multiple prey condition.

Navigating the empty flight room, single bats were able to explore the environment freely, without needing to search for prey, or avoiding obstacles within the flight space. Their flight path distributions in this environment broadly covered the available space, and were not concentrated on a specific point in the room (Figure 3, top

left). By contrast, when a single prey item was introduced into the empty room environment, bat presence heavily increased around the prey location (Fig. 3, top middle). Interestingly, when multiple prey items were available to the individual bat, no such high concentration of flight path distribution around prey locations was detectable. Instead the distribution of flight paths more closely mirrored that of the empty room – no prey condition (Fig. 3, top right). When individual bats navigated the forest environment, we saw a similar behavior: when no prey was present, bats perused the space freely, avoiding the tree obstacles, but showed no specific preference to any given place inside the flight room (Fig. 3, bottom left). By contrast, adding a single prey item caused the animals to focus their flight toward prey location(s) (Fig. 3, bottom middle).

**Figure 6. 3. Heat map distribution for single bat data across conditions.**




Top: Flight path distributions in the empty room conditions for different prey availabilities. Bottom: Flight path distributions in the forest conditions for different prey availabilities. Red dots indicate prey locations; green circles indicate tree locations across data collection days. All plots are normalized. High concentration at top middle/right corners show locations at which two bats preferred landing.

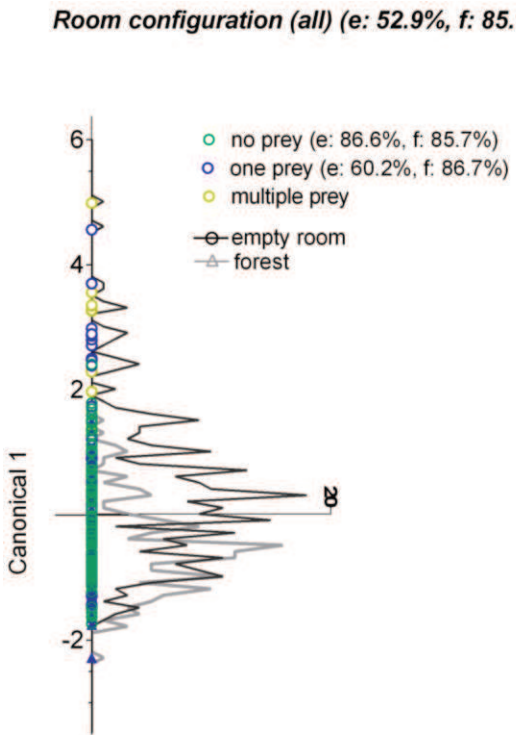
When bats navigate a cluttered environment or encounter targets of interest, such as prey, they adjust their echolocation behavior (Falk et al., 2015; Warnecke et al., 2015). For example, prey or close obstacles will cause the bat to buzz, in preparation for capture, landing or collision avoidance. We analyzed buzzing behavior across different environments and prey conditions, and show that bats buzzed significantly more often and made longer buzzes in conditions with prey present, compared to conditions without prey (buzz number: no prey: mean = 0.3 buzzes/trial, SEM = 0.03; some prey: 1.13 buzzes/trial, SEM = 0.06; $t = 7.95$, $p < 0.0001$; buzz length: no prey: mean = 50.6 ms, SEM = 12.6; some prey: 269 ms, SEM =15.4; $t = 10.93$, $p < 0.0001$). No difference of buzzing behavior across environmental conditions was observed ($F_{1,64} = 1.6$, $p = 0.2$).

As bats moved from navigating an open space to a more cluttered environment, they significantly decreased their call duration (empty: mean = 2.21 ms, SEM = 0.09, forest: mean = 1.8, SEM = 0.09, $F_{3,17} = 50$, $p < 0.0001$), and post-hoc analyses revealed that this decrease was only significant for environment ($p < 0.0001$), but not for prey availability ($p = 0.38$). However, prey availability significantly affected pulse interval and call rate, causing a decrease in PI (no prey: mean = 70.2 ms, SEM = 2; some prey: mean = 60.7 ms, SEM = 1.7, $F_{1,24} = 12$, $p = 0.002$) and an increase in call rate (no prey: mean = 14.3 calls/s, SEM = 0.43; some prey: mean = 15.9 call/s, SEM = 0.37; $F_{1,24} = 7.35$, $p = 0.012$) as prey was introduced. Note that buzz calls were excluded from the analysis, so the change in parameters reflects non-buzz related calls only and is comparable across conditions.

To learn whether call features were consistently changed depending on the environment or prey availability, we analyzed raw call features using a quadratic

discriminant function analysis (DFA). Using a stepwise function and adding start/end frequency, duration, number of buzzes, length of buzzes, and call rate as predictor variables to classify the environment (empty, forest), we found only a small reliability in these variables predicting the environment. Figure 4 illustrates that the DFA was able to classify about 64.8% of calls from a specific environment correctly. However, the model that resulted from the stepwise DFA showed that across all gender and prey availability options it was enough to include duration and end frequency as variables for a 64.8% correct classification of the data. In other words, adding an additional 6 variables (start frequency, duration, pulse interval, number of buzzes, length of buzzes, call rate) did not improve the classification.

**Figure 6. 4. Room classification for single bat data.**

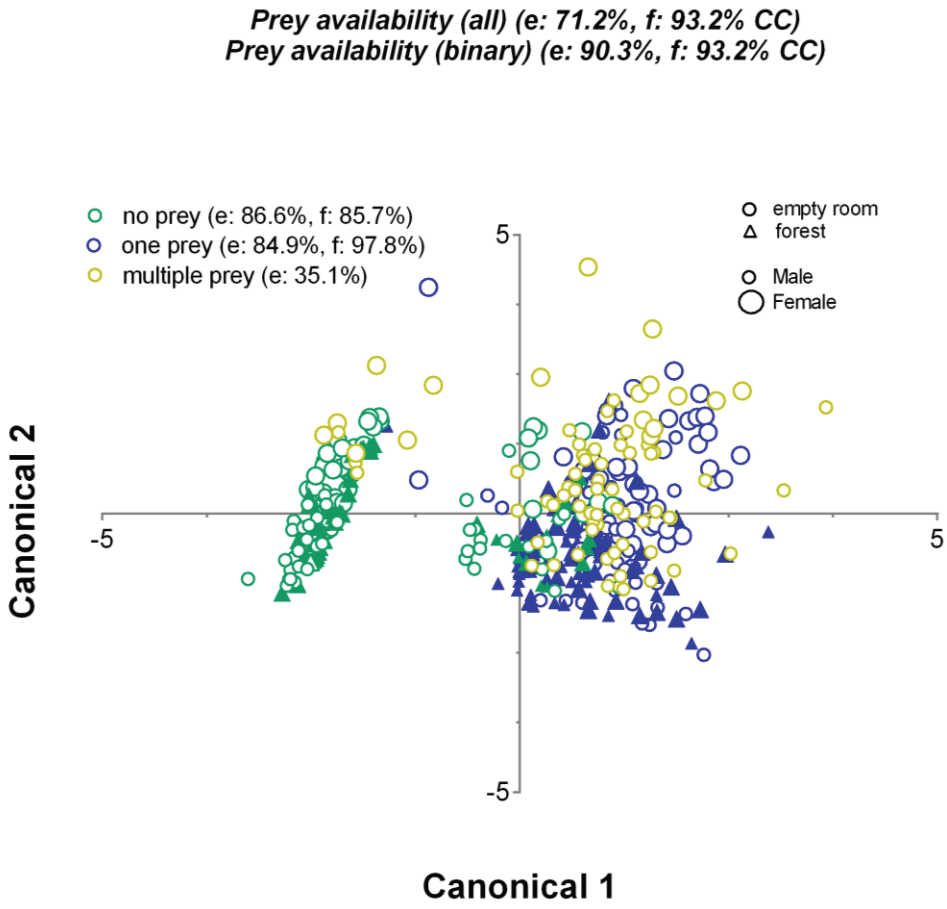



Graph plots the results of a quadratic DFA for classifying a given set of call features as belonging to either the empty (open circles, grey line) or forest (filled triangles, black line) condition. Overall, 64.8% of trials were correctly classified, with a misclassification rate of ~ 47% for all empty trials and ~15% for all forest trials. The respective results for room classification by prey availability is indicated by color. No difference was found between the classifications across prey availability.

Splitting the data by prey availability (no prey, single prey, multiple prey) helped the classification significantly: In trials with no prey, the correct classification of environment increased to about 79% using all variables (start/end frequencies, duration, PI, number of buzzes, length of buzzes, call rate). Similarly, environment was classified correctly 74% of the time in single prey trials using only end frequency, duration and PI. Including all variables did not change that prediction.

Prey availability changed flight path distributions and PI patterns, and as such we were interested in whether bats' call features and call behavior (buzzing, call rate) predicted prey availability. As stated above, availability of prey drastically increased the number and duration of buzzes, and we therefore hypothesized that call duration, PI and buzzing behavior might reliably predict prey availability. Figure 5 illustrates that the DFA model was able to reliably predict prey availability 76.8% of the time. Interestingly, empty room trials were classified incorrectly more often (28.8%) than forest trials (6.8%). Figure 5 also illustrates that trials with no prey (green) separate clearly from trials with single (blue) or multiple prey (yellow), but that trials in which prey is generally available do not separate out well. When we re-ran the classification using a binary prey availability (prey vs. no prey), correct classification jumped to 89.4%. In other words, we were able to correctly predict whether a specific trial in this data set was collected when there was prey present or not almost 90% of the time. The variables driving this classification were number of buzzes, end frequency and call duration, in that order.

**Figure 6. 5. Prey classification for single bat data.**

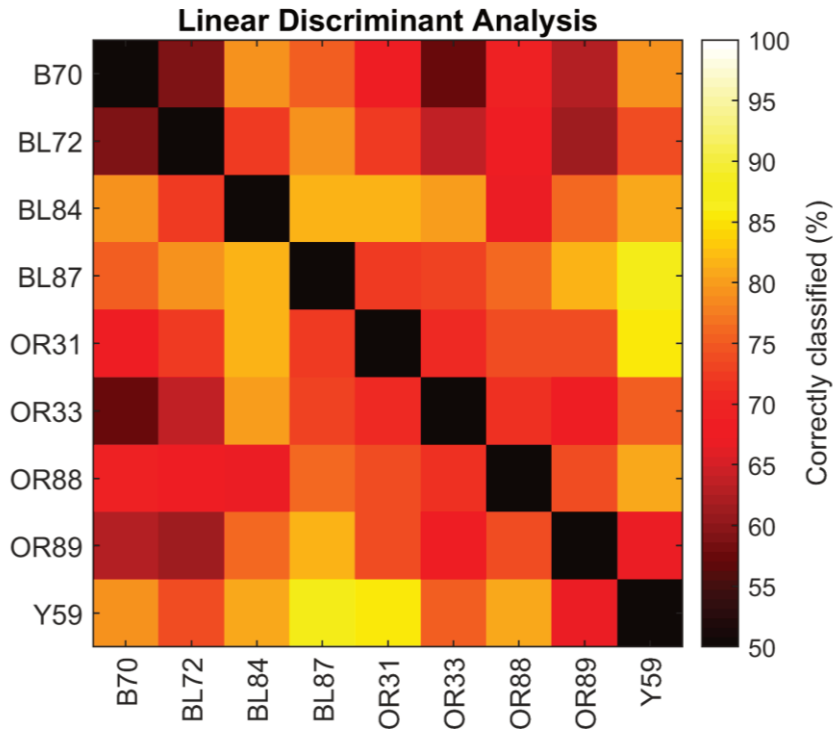



Graph plots the results of a quadratic DFA for classifying a given set of call features as belonging to either the no prey (green), single prey (blue) or multiple prey (yellow) condition. Empty trials are indicated by open circles; forest trials are indicated by filled triangles. Gender of bat per trial is indicated by marker size. Overall, 76.8% of trials were correctly classified when we separated prey into its three categories (no prey, single prey, multiple prey), and the classification increased to 89.4% correct when we categorized prey availability binary (no prey vs prey). No difference was found between environment or gender.

Chiu et al. (2008) were able to use call feature data from bats flying by themselves to predict the changes paired bats made to their calls when flying together, and to predict silent behavior in paired bat trials. In order to predict which bat pairs might show silent behavior, we calculated call feature similarity for all possible bat pairs from calls emitted in the empty room – single prey condition. This condition was chosen because it serves as the baseline for the paired bat data in which silent behavior would be

expected. Figure 6 illustrates a matrix of call similarity classification for all bat pairs, with lighter colors representing higher correct classification of call features for a given pair, and darker colors representing higher misclassification of call features for a given pair. For the linear discriminant function analysis, correctly classifying two or more classes (bats) means that the underlying values for each class were more different. In other words, a high classification of bat pair means that the call features of the two bats were very different; a low classification of bat pair means that the call features of the two bats were very similar. On average, the classification of bat pairs is about 73% correct, but individual values extend to as low as 57%, and as high as 87%. Classification of bats within bat pairs is dependent on how different the features of each bats calls are.

**Figure 6. 6. Call similarity matrix for all bat pairs from single bat data.**

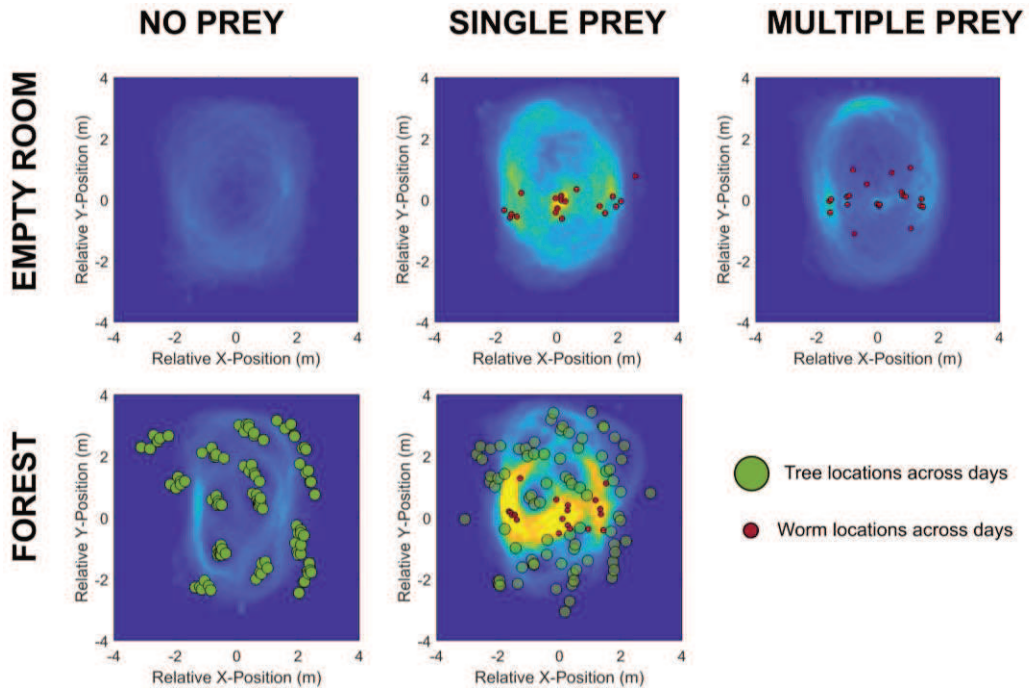



Heat map shows the percent correct classification (colorbar) of each bat within a bat pair (x-axis, y-axis) given call features extracted for each bat (see Fig. 2C). Lighter colors indicate better classification, while darker colors indicate worse classification. Male bats have a bat ID starting with "B"; all remaining bat IDs belong to female bats.

*Paired Bat Data*

Overall, the raw flight path data for paired bats generally mirrored the flight behavior of single bat flight paths: trials which contained no prey items showed a broad and consistent distribution of flight paths (Figure 7, top left), while the presence of a single prey item focused bat flight paths towards prey locations (Fig. 7, top middle). Further, as in single bat trials, multiple prey items appeared to dilute the flight path distributions, rather than show focused points at each "hub" of possible prey location (Fig. 7, top right). Flight paths in the forest environment showed similar patterns of broad and even distributions when no prey items were present (Fig. 7, bottom left), and stark concentration of bat presence around locations of prey items otherwise (Fig. 7, bottom middle).

**Figure 6. 7. Heat map distribution of paired bat data across conditions.**

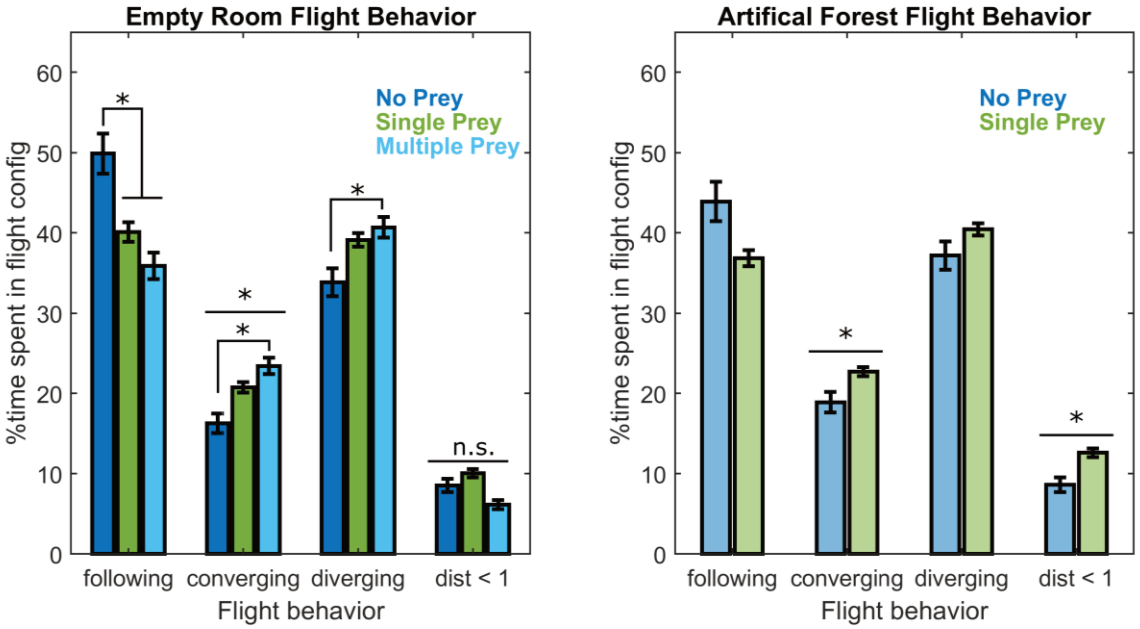



Top: Flight path distributions in the empty room conditions for different prey availabilities. Bottom: Flight path distributions in the forest conditions for different prey availabilities. Red dots indicate prey locations, green circles indicate tree locations across trials and data collection days. Tree locations for Forest, Single Prey are semi-transparent to facilitate viewing flight path distribution. All data are normalized.

These data allude to different flight configuration patterns that can be evaluated across conditions. As pairs of bats navigate their environment, they can engage in one of three flight configurations: following flight, converging flight, or diverging flight. Figure 8 plots the amount of time (y-axis) paired bats spent in a specific flight configuration (x-axis) in both the empty room (left) and artificial forest (right) environments. In empty conditions, when there was no prey in the flight room, bats spent about 51% of their time in following flight, compared to converging or diverging flight, which they engaged in 32% and 15% of the time, respectively (dark blue). As prey was introduced into the empty room environment, following flight decreased to about 40%, while both converging and diverging flight increased to 20 % and 39%, respectively (green). Increasing the amount of prey further increased these flight configuration patterns (light blue), but the amount of change from the single prey condition to the multiple prey condition was not significant. Overall, there was a significant effect of flight configuration ($F_{3,6} = 19$, $p = 0.0018$), showing that bats spent generally the least amount of time in converging flight patterns (mean = 19.9%, SEM = 3.57%), followed by diverging flight patterns (mean = 37.8%, SEM = 3.57%) and mostly pursued following flight (mean = 42.2%, SEM = 3.57%). Post-hoc analyses confirmed that amounts of following flight and diverging flight did not significantly differ from one another, but that both differed from the amount of converging flight (diverging-converging: $t = -3.52$, $p = 0.04$; following-converging: $t = -4.4$, $p = 0.017$). In addition, Figure 8 (left) shows that across all prey availability conditions, bats spent only very small amounts of time at distances closer than 1m (mean = 8.57%, SEM = 3.57%), and there was no difference across different prey availabilities.

When bats flew in the artificial forest, the flight patterns observed were similar to those in the empty flight room (Fig. 8, right), and we saw no significant changes across prey conditions. While the amount of time bats spent in different flight configurations when flying in the artificial forest closely mirrored that of the empty flight room, they were generally reduced (following flight: mean = 39%, SEM = 1.2%; converging flight: mean = 22.32%, SEM = 1.2%; diverging flight = 38.66%, SEM = 1.2%).

**Figure 6. 8. Time spent in different flight configurations across conditions.**

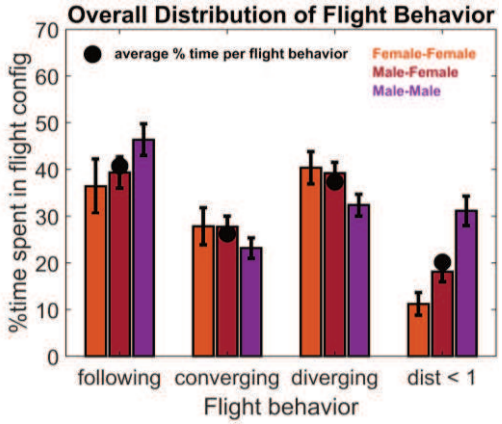



Left: Bar graph representing amount of time (y-axis) paired bas spent in a given flight configuration (x-axis) while navigating the empty room. Right: Bar graph representing amount of time (y-axis) paired bas spent in a given flight configuration (x-axis) while navigating the artificial forest. Prey availability conditions are indicated by color (see legend).

Warnecke et al., (2015) suggested that silent behavior might be related to the flight behavior of paired bats, and base their hypothesis on the data presented by Chiu et al., (2008), who show that silent behavior is especially prevalent in following flight and when bats are within 1 m of one another. Warnecke et al. (2015) further elaborate that

male bats were the ones driving the following flight behavior, while female bats tended to converge and diverge. To gain more insight into the amount of time bats of either gender spent in a given flight configuration, we analyzed the flight behavior in the empty room, single prey condition by gender combinations of each bat pair. Figure 9 plots the amount of time bat pairs spent in a particular flight configuration by gender pairing. Analyses confirmed that while the majority (~ 46%) of following flight in the empty room was driven by male pairs, there was no effect of paired genders, so the difference between pairs was insignificant. As reported above, there was an effect of flight configurations ($F_{2,43} = 8.1$, $p = 0.006$), and post-hoc analyses showed that the amount of time spent in converging flight differs from both following and diverging flight (see above). It is interesting to note, however, that male-male pairs spent about 32% of their time at distances closer than 1 m, in comparison to female-female of male-female pairs, who spent only about 10% to 18% at similar distances, respectively. Note that the data in Figure 9 come from trials which have been analyzed for their audio data, and thus present a subset of the empty room, single prey data set plotted in Figure 8.

**Figure 6. 9. Time spent in different flight configurations across gender pairs.**

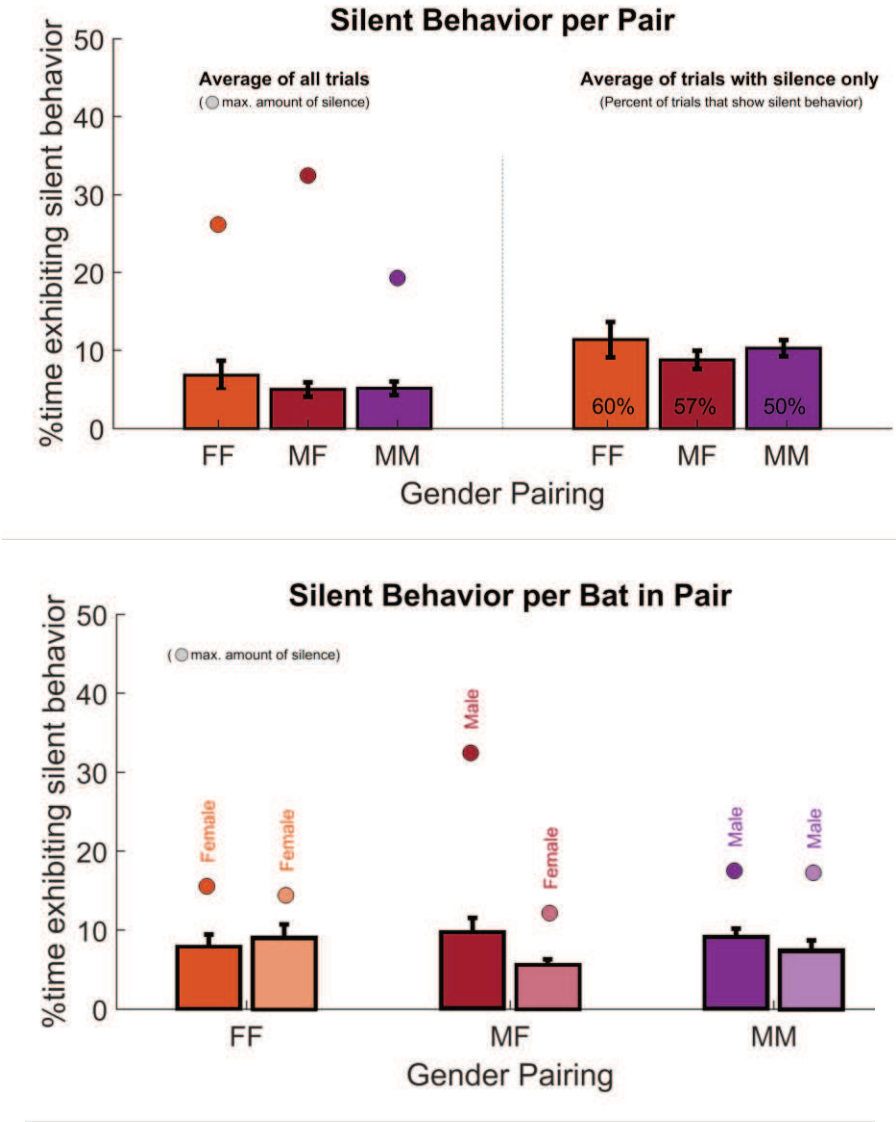



Bar graph representing amount of time (y-axis) paired bas spent in a given flight configuration (x-axis) while competing for a single prey item. Gender is indicated by color (see legend). Back circle represents mean per flight configuration.

About 40% of the time bats were following each other when they competed for a single prey item in the empty flight room (Figs. 8, 9). Further, ~ 20% of the time flight behaviors were executed at distances closer than 1 m. This suggests that we could expect silent behavior in this condition as previously described by Chiu et al. (2008). We calculated silent behavior as the percentage of time either bat did not emit sounds for at least 200 ms of the entire vocal time. For this data set, we report little amount of silent behavior across bat pairs. Figure 10 plots the amount of silent behavior for each pairing of gender, and illustrates that across all trials we observed about 8% to 10% silence per gender combination (Fig. 10, top). At most, in a single trial, a bat was silent for 27% of the time in female-female trials, 32% of the time in male-female trials, and 19% of the time in male-male trials. In a second analysis, we excluded trials without silent behavior (Fig. 10, top, right panel). Across different gender pair combinations, 50% to 60% of trials showed some amount of silent behavior, however, the average amount of silence never exceeded 12 %.

Warnecke et al. (2015) hypothesized that male bats may be driving silent behavior in paired trials. Thus, we analyzed the silence data split by each bat in a pair. Figure 10 (bottom) plots these data per gender of each bat in a pair. The bar graph shows that silent behavior in same-gender pairs was equal among the two bats in the pair, but tended to be driven by male bats in gender-mixed pairs (Fig. 10, bottom, red).

**Figure 6. 10. Silent behavior across bat pair gender combinations.**

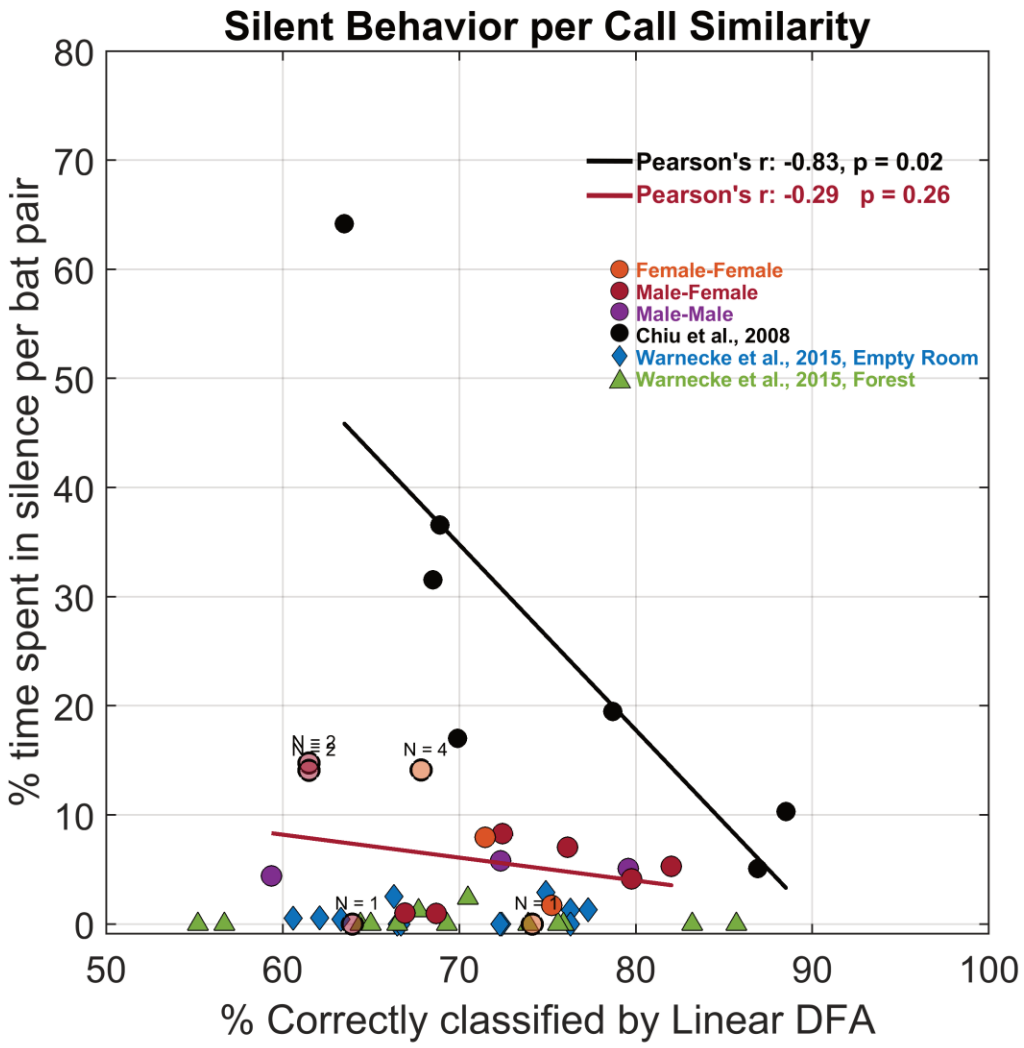



Top: Right Panel: Average amount of silent behavior (y-axis) across paired gender (x-axis) for all trials. Filled circles indicate maximum amount of silent behavior encountered in that gender combination. Left Panel: Average amount for silent behavior (y-axis) across paired gender (x-axis) for only trials that had silent behavior. Percentage in bar represent the amount of trials that did show silent behavior per gender combination. Bottom: Average amount of silent behavior (y-axis) across different genders within a bat pair (x-axis). Filled circles represent maximum amount of silence produce by gender of that bat.

Chiu et al., (2008) showed that the amount of silent behavior was not just dependent on flight behavior, but also on the amount of call similarity that paired bats had. Baseline call similarity was calculated from call features of single bat trials in the

empty room, single prey condition (see Methods, Fig. 6). Figure 11 plots the amount of silent behavior across call similarity, as classified by liner DFA, with higher values indicating low call similarity and lower values indicating high call similarity. As a reference, we also plotted the data from Warnecke et al. (2015) (green, blue) and Chiu et al., (2008) (black). Data from the present data set (colored circles) do not fall along the expectation first introduced by Chiu et al. (2008) (black line), but rather distribute unevenly below the 20%-line. Note that the three data points showing the largest amount of silent behavior are averages of two to four trials in a given bat pair; additional data will likely decrease these points' values to be similar to the remaining data of this dataset. Warnecke et al. (2015) hypothesized that silent behavior may be related to gender, as all of their bats were female and no silent behavior was found, and the bat pair showing the lowest amount of silent behavior in Chiu et al. (2008) was also a female-female pair. Additionally, the bat pair with the largest amount of silent behavior was a male-male pair in Chiu et al., (2008). Female-female pairs in the present study are indicated by orange circles, which fall within the limits of both previous data sets; male-male pairs are indicated by purple circles and show between 4% to 8% silent behavior.

**Figure 6. 11. Percent silence across call similarity.**




Scattered are percent silent behavior (y-axis) across correctly classified DFA (x-axis) for data from Chiu et al. (2008; black), and Warnecke et al. (2015; green and blue) along with data from the current experiment. Gender is indicated by color (see legend). All bats in Warnecke et al. were female, the single female-female pair in Chiu et al. is at 5% silence. Current data (colored circles) and data from Warnecke et al. (2015) do not match prediction made by Chiu et al., 2008; black line).

## *Discussion*

In the present study we aimed to understand how the flight and echolocation patterns of the echolocating big brown bat change as the single bat moves through

different environments, and as paired bats have to forage among other conspecifics. In that capacity, we aimed to improve our understanding of "silent behavior", its driving mechanism and function.

Echolocating bats dynamically adapt their flight and echolocation behavior when they encounter clutter or pursue a target of interest. For example, they change the amplitude, duration and frequencies of their calls as they approach objects, and lock their sonar beam onto the target of interest in the last moments before capture (Griffin, 1958; Kalko and Schnitzler, 1989; Hartley, 1992; Falk et al., 2011; Ghose and Moss, 2003). When individual bats navigated different environments in this study, we observed similar behaviors: bats decreased their call duration in cluttered environments, but the presence of prey in general did not affect call duration. This suggests that even multiple prey items do not "clutter" the space in a manner similar to having several larger objects scattered throughout an otherwise empty space. As expected, the amount of prey significantly influenced buzzing behavior of the bat. When prey was available, bats buzzed more often, and for longer durations, though we did not observe a difference in buzzing behavior between single prey and multiple prey conditions. This is to be expected, because in the multiple worm conditions a trial was triggered when at least one bat had caught prey. As such, unless either of the bats had caught or attempted to capture another prey within the preceding 5 seconds, only one buzz would be detected in the recordings. Even when no prey was present, bats did occasionally buzz in the empty flight room, most commonly when they were getting close to a wall or prepared to land. Overall, we can conclude that call duration is mostly affected by the amount of clutter within the immediate environment, while buzzing behavior is affected by the amount of prey.

Given that both environment and prey availability showed significant differences for some echolocation behaviors, we were interested in whether we could predict, with just one average value per trial, whether that trial was collected in a specific environment or prey condition. Using a quadratic discriminant function, we found that classification for the type of environment was low (Fig. 4, ~ 65%). However, the same classification improved significantly when the data were split by prey condition, which suggests that the echolocation behavior in each of the prey availability conditions may have been stereotyped, making the classification more stable. When the same classification method was used to see if we could classify the type of prey availability, we saw that the no prey trials separated clearly from the single and multiple prey trials (Fig. 5), and overall, we were able to classify prey condition correctly about 80% of the time. Interestingly, trials in the cluttered condition were classified correctly more often than trials from the empty room condition, which suggests that call parameters in the empty room may varied more between bats and trials, so their predictive value was lower. By contrast, introducing clutter may have stereotyped the call behavior across individuals and trials to produce a consistent data set, that could be classified more reliably. Additionally, single and multiple prey items were not well separated from each other, which suggests that the echolocation behavior across those two conditions was fairly similar. This notion is supported by the fact that we did not observe significant changes between the single vs. multiple prey conditions for call duration or buzzing behavior, or flight tracks between these conditions.

Chiu et al. (2008) showed that silence occurred when paired bats competed for a single prey item in an open room, but how would silence be affected when the environment is cluttered, or when there is no prey or competition? In an effort to understand more about the factors that influence silent behavior, Warnecke et al. (2015) repeated Chiu et al. (2008)'s experiment, and extended it to another condition, having bats compete for a single prey item in a cluttered room. They hypothesized that silent behavior would decrease, if detectable at all, in the cluttered environment, as the bats would have to monitor locations of objects while they navigated that space. Indeed, they did not find silent behavior in the cluttered space, however, to their surprise, they also could not replicate data showing any silent behavior in the open space, as Chiu et al. (2008) had reported it. In-depth analysis of the data revealed two differences in the data sets by Warnecke et al. (2015) and Chiu et al. (2008): First, all of Warnecke et al., (2015)'s bats were female, while all but two bats in the Chiu et al. (2008) study were male. Second, Chiu et al. had reported that their bats were in following flight configurations about 65% of the time, and 30% of the time bats were also at close distances. By contrast, Warnecke et al.'s data showed only about 40% following flight behavior, and less than 10% of the time bats were closer than 1 m. These results suggest that silent behavior could largely be driven by the inter-bat distances, especially in following flight, or that gender might be a driving factor in which bat pairs show silent behavior. In support of that hypothesis, the single female-female pair that was tested by Chiu et al., only about 5% of silent behavior was observed.

The present data set aimed to understand the function of silent behavior and what factors drive it, by testing single and paired bats in a variety of conditions. When paired

bats navigated the empty flight room with no prey, they had not yet been trained to capture prey, and thus could not associate the flight room with food. As such that condition tested if silent behavior is elicited by the presence of another bat. If silent behavior was observed only in that condition, it would favor the hypothesis that its function might be a cooperation between the two bats to reduce acoustic reverberation. By contrast, if silent behavior was observed only when paired bas competed for a single mealworm in an open space, it would suggest that competition for food drives silent behavior, and that a bat might be "eavesdropping" on another bat's calls to increase food uptake. As a third option, foraging in an empty flight room in the presence of another bat, while surrounded by several prey items eliminates the competitive component of the single prey condition, but keeps the foraging aspect. Thus, if silent behavior was observed in this condition, and possibly also in the single prey condition, it might suggest that general foraging is subject to silent behavior. Naturally, independent of the condition, we would expect that bats with greater call similarity showed generally more silent behavior, and that silent behavior would largely occur during following flight configurations. All of these variations of prey availabilities were further investigated in a cluttered environment, and we generally expected silent behavior to be low or absent in any of those conditions.

Chiu et al. (2008) used a linear discriminant analysis function to estimate call similarity across different bat pairs from baseline call features, and used those data to predict silent behavior per bat pair. In this study, we calculated baseline call similarity in the same way, to be able to directly compare our results to Chiu et al.'s data. Overall, we find that a linear DFA can classify the call features of two bats correctly about 73% of the

time, but individual values go as low as 57%, and reach as high as 87%. Given Chiu et al.'s correlation between call similarity and silent behavior, we would expect as much as 80% to 90% silent behavior in bat pairs with high call similarity (DFA: 57%, pair B70 – BL72; Fig. 6), and as little as 5% to 8% silent behavior in bat pairs with low call similarity (DFA: 87%, pair BL87 – Y59; Fig. 6). However, the largest amount of silent behavior we observed in a bat pair was ~ 15% for a call similarity rating of 62% correct, which Chiu et al. (2008) proposed should render about 50% silent behavior (Fig. 11). Importantly, this bat pair's silence estimate stems from two trials, and it is likely that additional trials may lower the amount of silent behavior for that pair, to more closely fit in with the remaining 14 data points, most of which are scattered between 0% to 10% silent behavior independent of their baseline call similarity.

Chiu et al. (2008) reported that call similarity was a deciding factor in the amount of silent behavior observed in their data set, and Warnecke et al. (2015) expanded that finding by hypothesizing that gender may be another contributing component. To address this hypothesis, we used 4 male and 5 female bats in this study, which created 3 male-male pairs, 4 female-female pairs and several gender-mixed pairs. Figure 11 indicates the bat pair's gender combination by color. If gender was related to the amount of silent behavior exhibited, we would expect female-female pairs (orange) to have lowest amounts of silence, and male-male pairs (purple) to show largest amounts of silence. However, the genders of bat pairs appear mixed across amounts of silence. In fact, we see that the three male-male pairs' call similarity estimates span a range from 59% to 79%, which, given previous reports, should produce 80% to 12% of silent behavior. Yet, all male-male pairs showed less than 8% of silence (Fig. 11).

As a third factor, both Chiu et al. (2008) and Warnecke et al., (2015) noted that flight configuration of paired bats is another indicator of silent behavior. Chiu and colleagues first reported that silence was mostly seen during following flight at close distances, and Warnecke and colleagues discussed that their data set showed little following flight and almost no flight at close distances. They mention that it is impossible for them to separate gender out of the flight behavior since they had only female bats, however, they were able to analyze Chiu et al.'s original data set and noted that in gender-mixed pairs it was consistently the male bat who followed the leading female bat. Warnecke and colleagues therefore hypothesized that female bats may generally display less following flight compared to males, and prefer to converge and diverge, or fly in opposite circles and figure-8 configurations. In our data set we analyzed the amount of time that a given pair spent in any of the three flight configurations while competing for a single prey item in the open room (Figs. 8, 9). While we do see that male-male bat pairs spent more time following each other than female-female pairs did, the difference was not statistically significant. Overall, however, male-male pairs did spend a considerable amount of their flight (~ 32%) at distances closer than 1 m, which, given previous reports, leads to an expectation of silent behavior (Chiu et al., 2008). Again, no expected amounts of silent behavior were found (Fig. 11). Female-female bats showed flight at close distances only about 10% of the time, a finding that supports Warnecke et al.'s reports of flight data, but is unlikely to explain the little amount of silent behavior found for female-female pairs (~ 5%, Fig. 11).

In summary, previous work suggests that silent behavior is prevalent if the following three cases are true: (1) paired bats compete for a single prey item in an

otherwise empty flight space, (2) paired bats are in a following flight configuration at close distances, and (3) paired bats have similar call structures. It has further been hypothesized that male pairs are more likely to exhibit silent behavior than female bats, but it is possible that male bats generally spend more time following each other, chasing each other even, while female bats may choose not to do so. Investigating the relationship between these two factors, gender and flight configuration, is challenging, as neither can be easily controlled.

In our study, we recreated the environment in which silent behavior was first reported, and analyzed flight configurations and call similarity. Overall, we did not find any evidence that silent behavior was as prevalent as reported by Chiu et al. (2008): bats with highly similar call structures show 0% to 15% of silent behavior, and even though some bat pairs spent about 30% of their flight at close distances and following each other, no silence comparable to Chiu et al.'s results was replicated.

What could be the reasons for the discrepancy between these data sets? Below, I will outline several arguments that can explain the differences between the previous and current data.

1. In the current data set, all sounds that were emitted by the echolocating bat during a trial were counted, independent of whether they were social calls or echolocation calls. Previous work shows that some bat pairs, mostly male-male bat pairs, produce "food claiming" calls when they compete for prey (Wright et al., 2013), a kind of social call to possibly indicate to a buzzing bat that another individual is also interested in the prey. Since any bat-produced sounds interrupt (deliberate) silence, we decided that all bat-produced sounds would be counted as a call. It is unclear whether Chiu et al. (2008)

included social calls in their analysis, but not doing so could create silence where there was none.

2. Collecting data is a noisy process, and it is possible that for a given trial the flight path is split into several segments, because one or both of the bats exited the area that can be effectively re-constructed in 3D using IR motion-tracking. In the present data set, we only analyzed calls that were emitted when both bats' locations were detected, and excluded any calls which occurred during times at which only one or no bat's flight path could be re-constructed (e.g. Fig. S2). This was a necessary step in our data processing, as call assignment was made by doing a time-difference-of-arrival calculation. In other words, if there was no position for one of the bats, it is impossible to say with confidence that a call is indeed from a specific bat, since the "out of view" bat could be at a location where this call could also have arrived at the designated time. From personal conversation with the first author of the Chiu et al. (2008) study, and re-investigating their data, we learned that they also assigned calls when only one bat's track could be reconstructed. In such situations, they based their call assignments on subjective classification of call structure. For example, Figure S1 (C) shows that it is possible to visually tell the difference between two bats' calls using the spectrogram of the sound recording. However, in the present data set we have seen evidence that bats change their call structure in the course of a trial, a finding which Chiu and others have also published in 2009 (Chiu et al., 2009). This means that call structures between two bats can be confused (Fig.S1(B)). As such, given that Chiu et al. (2008) assigned calls without always having both bats' flight paths, it is possible that call assignments were mislabeled. This can create a silence period assigned to a bat which did not actually cease to

echolocate. Further, this can change the call features connected to a given bat's calls (see below).

3. As discussed before, Chiu et al. showed that silent behavior is correlated with call similarity. Chiu et al. extracted call feature measurements by manually setting markers at start and end time/frequency estimates. These estimates are subject to the signal-to-noise ratio (SNR) of the channel they are marked in and subjective labeling techniques. Personal conversation with the first author, Chiu, revealed that when the SNR was low, call features were marked where the authors estimated the call should have started/ended given previous call markings of that bat. This can lead to considerable variability of call feature extraction across calls, individuals, and trials, which can affect the call similarity computations presented in correlation to silent behavior. In the present study, we use an automatic MATLAB code to extract call features based on peak frequency of each call. Further, call features for each call were extracted in the channel that had the best SNR for that specific call. As mentioned in this chapter's *Materials and Methods*, we manually inspected each call feature after automatic marking and corrected about 13% of markings, meaning that 13% of calls have a subjective influence in their marking; most commonly, start times, but not frequencies, were mis-assigned. Recent work discusses the extent to which natural causes, such as spreading loss and spherical attenuation, and technology may misrepresent actual frequency information of bat echolocation calls (Ratcliffe and Jakobsen, 2018), however, we argue that in comparison to Chiu et al.'s call marking procedure, the present approach has less subjectivity and thereby presents more accurate objective measurements.

4. When assigning calls, Chiu et al. (2008) marked calls in two channels and calculated which bat made that marked call given the microphones' and each bat's current locations. In their Supporting Information Chiu et al. (2008) explain that the accuracy of their call markings across two channels had to be less than 1 ms for inter-bat distances of at least 0.5 m, and less than 0.5 ms for inter-bat distances less than 50 cm. Chiu and colleagues set these call markers manually, meaning that placing a call marker at 1.0000 s would assign the call to one bat, but placing it at 1.0005 s would assign it to another bat. This process usually occurs when bats are at close distances. Such marking is very challenging and can be misplaced (Fig. S1(B)). With regard to this, it is important to note that most silent behavior was seen at close distances in bat pairs that have similar call structures. As such, calls may have been mis-assigned because (1) the inter-bat distance was too close to get a reliable marking on only two channels, and (2) the call structures were similar, so visual inspection was unreliable.

In our data set, we were able to use up to 20 channels of audio recordings. If bats were at very close inter-bat distances, TDOA calculations across all channels could reliably disambiguate which bat emitted a given call.

5. Most importantly, it is possible that Chiu et al. missed echolocation calls in their data processing. Chiu et al. (2008) used 3 ultrasonic microphones that were placed at different locations around the empty flight room. For most of their data analysis, they marked calls in two channels, and used a third channel only in situations where inter-bat distance was small. In the present data set, we collected data using 20 audio channels, 18 of which were mounted around the perimeter of the room, and 2 of which were at a lower elevation in the middle of the room. We always marked calls in one "floor channel" and

one “wall channel” as the difference in elevation between the two microphone locations helps to resolve ambiguity in call assignments when bats were at close inter-bat distances. We pre-assigned calls based on the markings in those two channels and compared predicted call time per bat in all remaining channels. During that process, it was not unusual for us to detect calls in another channel, which one of the bats was close to, that did not show up in either of the selected marked channels (Figures S2, S3). This situation could arise when the call of bat 1 was low in amplitude and masked by echo noise from bat 2’s call that was picked up by the microphone (Fig. S2), or when the microphone was too far from the call-emitting bat, and the sound had attenuated enough to not be picked up above the microphone’s noise threshold (Fig. S3). Overall, we estimate that we would have seen *at least* a 10% to 30% increase in silent behavior, had we not used the remaining channels to confirm echolocation patterns at specific times. We predict that this number might be even higher for the data in Chiu et al. (2008)’s data set, because bats spent more time at close distances.

We want to stress that it is possible Chiu and colleagues truly did not observe any calls from the silent bat in the time periods of silence they report. However, given our observations that some calls only show up in recordings of microphones that were close to the bat’s location, it seems likely that the “silent bat” in Chiu et al. (2008) may have actually emitted calls, but the experimental equipment did not allow for them to be picked up.

Summarizing the arguments above, Chiu and colleagues used the technology and equipment available to them at the time in an effort to show evidence for an “absence sounds,” a difficult task. We propose that the experimental equipment and availability of

a full bandwidth 32-channel microphone array allowed us to more carefully investigate "silent behavior", and discover that it might be less prevalent than previously reported.

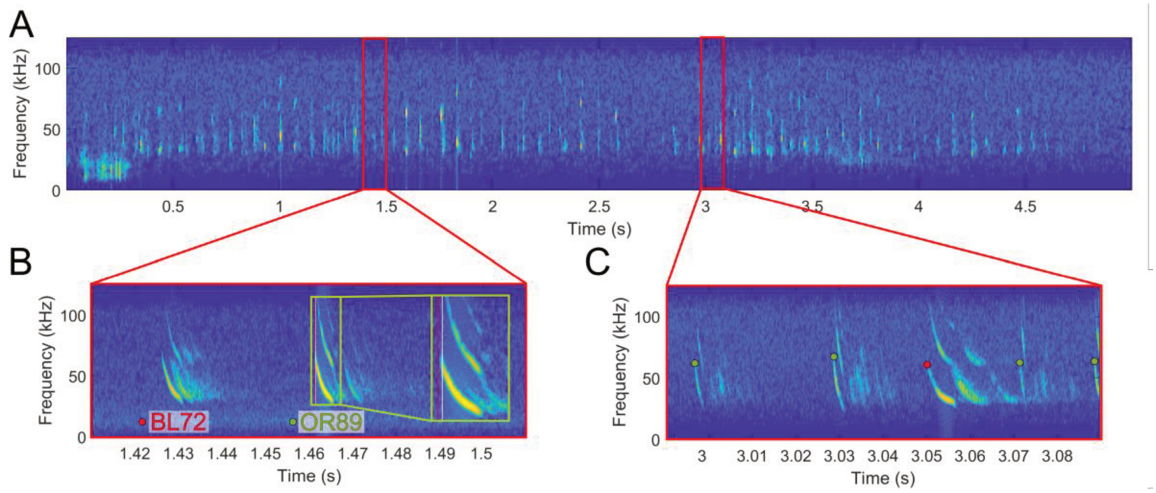
Echolocating bats emits calls up to about 120 to 140 dB SPL when flying in their natural environment (Surlykke and Kalko, 2008). While bats may not cease to echolocate completely, it is possible that they reduce the amplitude of their calls in competitive foraging situations, which could mean that less sensitive microphones might not pick up the signal, and a false silent period could be reported. It would be of interested to investigate this hypothesis by outfitting the bat with body- or head-mounted telemetry, a light-weight microphone and radio transmitter.

Despite analyses revealing prevalence of silent behavior below that reported by Chiu et al. (2008), we do see small amounts of silent behavior: up to about 15% of the time one of two bats ceased to echolocate for at least 200 ms; on average bats were silent for about 385 ms at a time. At a flight speed of 3.5 m/s, this means the bat flew about 1.35 m without emitting a call – though possibly listening to the other bat's signals and echoes. Silent behavior also occurs in single bat flights. Here, bats ceased to echolocate for an average of 245 ms, or ~ 85 cm of flight. 15% of all calls in the single bat – empty room – no prey condition occurred at PIs greater than 100 ms, 0.2% of calls were at PIs greater than 200 ms. By comparison, Chiu et al. reported that 0.08% of single bat trials showed silent behavior. Overall, this suggests that pulse intervals of 200 ms or greater were uncommon in single flight conditions, and that their increased occurrence during paired trials was no accident. However, given the results of both Warnecke et al. (2015) and the present study, silent behavior may be less common than previously reported.

*Supplementary Information*

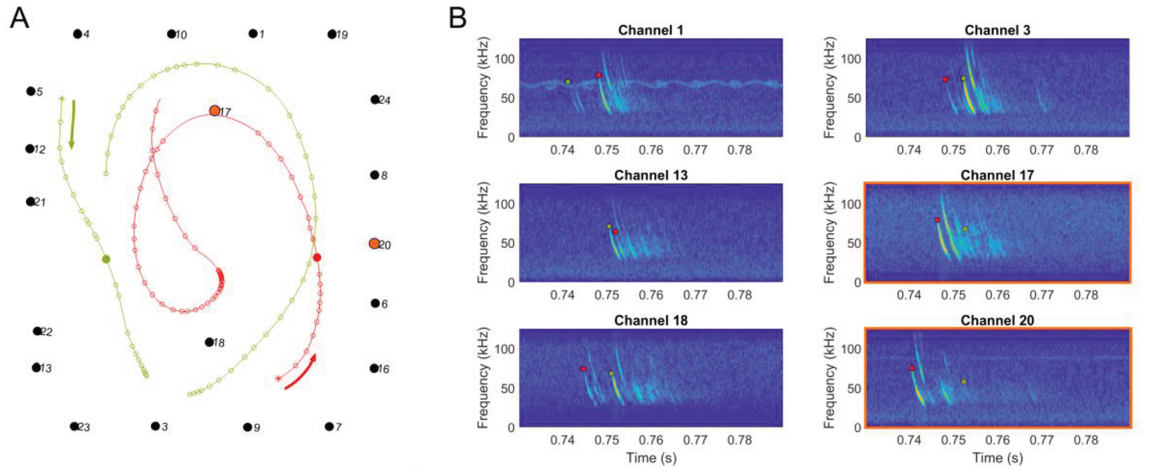
In the Supplementary Information, we are providing some figures of actual trials that show challenges relating to marking and assigning of calls in paired bat trials.

**Figure 6. 12. S1. Similarity of call structure and challenges in assigning calls.**



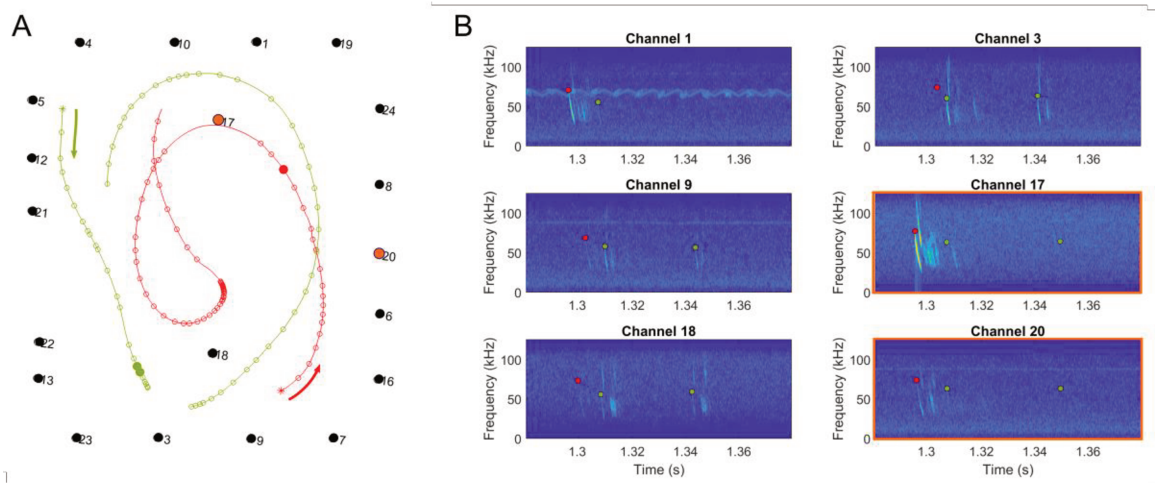

(A) Spectrogram of the entire 5-second trial. Indicated in red are time points at which call assignment challenges are found. (B) Expansion of 100 ms window at 1.41 seconds into the trial (see red outline in (A)). The first call around 1.425 s was assigned to BL72 (red), while the second call around 1.46 s was assigned to OR89 (green). Note the stark similarity in each bat's call structure. Visual inspection of these two calls might easily lead to mis-assignment of calls. Green rectangle expands call of OR89, to illustrate the small difference in time for a 0.5 ms difference in call assignment. If the marker was set at the red line, the call assignment would be for bat1, but if the marker was set closer to the white line, call assignment would be bat2. Manual marking for bats at close distances with only two channels can easily lead to mis-assignment. (C) Expansion of 100 ms window at 2.99 seconds into the trial (see read outline in (A)). Colors of call assignments match those from (B). Call features and structure of OR89 has changed dramatically, and visual assignment of calls is much easier in this scenario. Note that this stark change in call structure happen in just 1.5 seconds, and may occur at even smaller time spans.

**Figure 6. 13. S2. Example of calls embedded in echo noise.**




(A) Top down view of the flight room configuration for this particular trial, including microphones (black), and bat tracks of BL72 (red) and BL84 (green). Numbers next to microphones indicate channel numbers. Orange circles indicate channels that were marked for this trial (Ch17: Floor microphone, Ch20: Wall microphone). Arrows next to flight tracks indicate flight direction. Open circles indicate calls emitted by that bat, filled circles indicate calls labeled in (B). (B) Spectrograms of 6 channels for 70 ms surrounding the calls marked in (A). Chs 17 and 20 (orange) were the ones originally marked. Calls for BL84 (green circles) had not been detected originally. Only by auto-detecting and manually confirming calls in other channels, Chs 13, 18 and 3, which were close to BL84 (green) at call emission, did we add markings for the calculated time of those calls to Chs 17 and 20. Note that BL72 did *not* produce silent behavior (track part without circles). No calls are marked when the location of other bat, BL84, could not be detected (gap in green track). Calls are only marked when both bats are present, and "silence" due to gaps in tracks are excluded.

**Figure 6. 14. S3. Example of strongly attenuated calls.**




(A) Same as Fig. S2(A). (B) Spectrograms of 6 channels for 100 ms surrounding the calls marked in (A). Chs 17 and 20 (orange) were the ones originally marked. Calls for BL84 (green circles) had not been detected originally. Only by auto-detecting and manually confirming calls in other channels, Chs 3, 9 and 18, which were close to BL84 (green) at call emission, did we add markings for the calculated time of those calls to Chs 17 and 20.

# Chapter 7

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# General discussion

Natural environments are noisy. Whether I walk down St. Paul Street on my way to work, or am trekking through the rainforest on my vacation, my brain constantly analyzes incoming complex sound waves and helps me process and identify sounds of interest – in human communications such sounds are often speech signals. However, sound analysis is not special to humans. Most non-human animals make use of sound to inform themselves about nearby conspecifics, for example to identify mates, and to navigate. The nocturnal echolocating bat relies on sound for navigation and foraging, constantly needing to integrate and segregate information in echo signals to adapt its future movements.

This thesis examined how continuously changing sensory inputs in the echo-acoustic scene influence bat navigation and echolocation in cluttered environments. To understand the mechanisms that may aid the bat in analyzing its acoustic scene, we used two approaches: First, inspired by research on optic flow, we investigated the bat's adaptations to changes of controlled echo-acoustic flow patterns. Second, we extended previous research on competitive foraging in pairs, by investigating dynamic changes to flight patterns, and temporal patterning of echolocation calls.

An acoustically-guided animal, the echolocating bat, relies on hearing to process echo information from its surroundings. In a spatially complex environment, such as a forest

clearing, each sonar broadcast results in a cascade of echoes from trees at different positions relative to the bat. The arrival times and directions of echoes within the cascade change as the bat flies, resulting in dynamic echo flow patterns. In **Chapter 2**, I presented research that evaluated how different echo flow patterns influence bat flight and echolocation behavior. Briefly, when bats navigated through a corridor which returned different patterns of echo-acoustic flow to the bat, the results showed that bats consistently veered towards the corridor side built from sparsely-spaced poles at ~ 36 cm spacing. While these results gave a first indication of how flight behavior is impacted by manipulations of controlled acoustic environmental clutter, the underlying processes remained elusive.

What drives flight path selection in complex environments, and how are echo cascades represented in the bat brain? Results from Chapter 2 suggested that flight adaptations in echo flow corridors are either in response to the intensity of echo cascades, or in response to the timing between echoes within cascades. In **Chapter 3**, I combined behavioral analyses and neural recordings to discover that it is the interval between echoes which influences flight path selection by the bat. Specifically, during behavioral data collection I manipulated the reflectivity of corridor walls by reducing the intensity of returning echo cascades, and demonstrated that echo intensity did not influence flight path selection. Second, by manipulating the density of corridor walls, which changed the intervals between echoes within the echo cascades, I induced a significant change in the bat's flight trajectory. This suggested that the spacing between poles, or time between echo arrival times influenced bat flight behavior. Third, by recording local auditory evoked activity in the bat inferior colliculus in response to echo playbacks from different

density corridor walls, I discovered differential modulation of neural responses. Specifically, I found that neural response patterns follow separate echoes within a cascade only if the echoes are separated by *at least* 2 ms. This was a crucial discovery, because behavioral tests showed that bats flew *towards* the corridor wall which returned echoes separated by about 2 ms, and away from the wall that returned echoes separated by less than 2 ms. Collectively, these data suggest that the bat selects its flight path to "hear out" echo-acoustic patterns for flight guidance.

As humans and other animals navigate a 3D world, they rely on dynamic sensory information to navigate, forage, and avoid obstacles. Such natural behaviors invoke feedback between sensory space representation and action-selection (Lewicki et al., 2014). For example, studies of visually-guided animals have demonstrated that flight paths in flight corridors are adapted to balance optic flow across the two eyes (Srinivasan et al., 1996). While the two concepts, optic and echo flow, seem closely related, it is important to note several differences between them. In optic flow, the animal's own movement contributes directly to the experience of image motion (Gibson, 1950; Srinivasan, 2011), and if the animal and objects in its immediate surrounding were to remain stationary, no optic flow percept would occur. By contrast, a sonar emission by a stationary bat returns a cascade of echoes that is dependent on the distance and direction of the objects to the bat. Moreover, when the bat is in motion, it may still be receiving echoes from the previous call, while it is emitting its next call that elicits a series of new echoes, creating complex sound patterns. Such overlap of sensory stimuli is not experienced in visual animals during optic flow conditions, because the physics of sound and light differ.

A collection of studies has shown that adaptive flight behaviors in several visual species occur in response to different spatial frequencies of OF cues (e.g. Srinivasan et al., 1996; Dyhr and Higgins, 2010). These spatial frequencies are manipulated by changing the width of the black and white stripes used within the experimental corridor. By contrast, the manipulations in our echo flow environment increased the width of the space between poles, but poles themselves remained at a diameter of 2.5 cm. To achieve a different acoustic environment, we would have to increase the diameter of poles significantly, as even slightly bigger diameters will still return a point echo, as opposed to a surface echo, which might be stronger and cause greater echo overlap.

Optic flow has been investigated in a variety of visual species ranging from honey bees to budgerigars, which do not use stereoscopic vision to measure distances to objects or surfaces. Instead, they balance the apparent angular speeds of sensory inputs across their two eyes; both the left and right eyes contribute separate input from the left and right visual fields, respectively (e.g. Srinivasan, 1996; Bhagavatula et al., 2011). By contrast, while each ear of the echolocating bat contributes separate acoustic input, the sensory signal received at either ear is a merged sound wave of echo energy from both corridor walls, rather than just the echo cascade of the left or right wall. This merging of sound energy returning from the two corridor walls may be a driving factor in why the bats deviated toward the sparsely-spaced corridor side when they fly through the echo flow setup.

These differences between optic and acoustic flow might support the notion that visual animals balance the apparent image motion across their two eyes, while the

echolocating bat seems to be aiming to hear out a signal embedded in noise that it can use for guidance, instead.

In Chapter 3 we discuss that the echolocating big brown bat may represent the densely-spaced corridor wall as a single, noisy object, because echoes within the echo cascade return at intervals that are too close in time to be resolved. A similar argument has been made by Dyhr and Higgins (2010) with respect to flight adaptations in the honeybee: their results show that an increase of spatial frequency in corridor grating caused bees to adapt their flight paths in the same way they did when navigating a corridor with uniformly gray walls. The authors briefly mention that the bee might not be able to resolve the gratings at high spatial frequencies and may therefore perceive them as a gray wall. However, they note that this hypothesis does not account for the slower flight speeds that is observed when bees navigate uniformly gray wall conditions. This point is of little concern for our data, as flight speed did not change across conditions, and is independent of the interval between echoes returning from corridor walls.

Despite these new insights into the impact of different echo-acoustic flow patterns, there are still aspects about the intricacies of how echo flow influences bat flight that remain unclear. For example, our data suggest that in the echo flow environment, echoes within cascades should return at intervals of at least 2 ms to be resolved as distinct objects, and guide flight. However, is this an absolute minimum needed for flight guidance, or does it adapt dynamically as the echo scene of the dense or sparse corridor wall is manipulated? Given our findings (Chapter 3), and Sanderson and Simmons' results that auditory evoked responses recover to full amplitude after ~ 2 ms (Sanderson and Simmons, 2000), it suggests that the 2 ms interval between echoes in this specific

environment is the minimum interval for bats to guide their flight by. Our behavioral data tested bat flight deviation at 12 cm and 36 cm pole spacing. If a 2 ms interval between echoes is the absolute minimum interval that guides flight in the echo flow corridor, gradually increasing the spacing between poles from 12 cm to 36 cm should not alter the flight path selection until both corridor sides have 36 cm spacing. At that point, the bat will center its flight path (Fig. 3.2)

Further, it will be interesting to evaluate whether longer echo intervals change flight guidance. For example, given our behavioral results (Fig. 3.2), I would hypothesize that increasing the 36 cm spacing between poles on the sparse side, while keeping the dense side at 12 cm spacing, should not change the overall flight deviation. This claim is based on behavioral data from the condition in which the bat navigated a corridor whose walls were built from sparse pole spacing and a felt wall. In that condition the bat centered its flight path (Fig. 3.2), presumably because it did not need to deviate in order to hear out a resolvable pattern. Given this reasoning, any imbalanced conditions of dense (12 cm) and sparse (36 cm or greater) pole spacing should induce the same amount of deviation in the bat flight path, a deviation that allows it to hear out a resolvable echo pattern. However, if the bat's flight deviation from the midline were to increase as the spacing of the poles on the sparse side increases, the 2 ms interval between echoes may be the minimum interval, but larger intervals might mitigate flight guidance even more.

Another interesting question would be to investigate whether the effect of echo flow is relative: how does the bat's flight path change, as we increase the spacing of poles on both the sparse and dense corridor walls, such that their relative spacing stays the same, but the absolute spacing increases? One challenge in this setup could be that bats may

exit the corridor by flying through the gaps between sparsely-spaced poles as they extend beyond ~ 45-50 cm, but if the animals continue to veer towards the side with wider spacing between poles, and then center themselves when the dense side approaches 36 cm spacing, it would give additional strong behavioral evidence for a minimum threshold of echo interval within cascades that guide flight path selection.

Different species of echolocating bats have evolved a variety of echolocation signals and it is of interest to understand how these different species might adapt their echolocation and flight behavior in response to the same environment. In **Chapter 4**, I presented behavioral data of the Taiwanese leaf-nosed bat, *Hipposiderid armiger terasensis*, navigating through the same echo flow environment discussed in Chapters 2 and 3. Our results indicate that the two species showed different flight and echolocation patterns: while the big brown bat, which emits short broadband FM signals deviated towards the sparse corridor side, the Taiwanese leaf-nosed bat, which uses comparatively long, narrowband CF signals, did not. The data set presented in Chapter 4 was motivated by discussions about the effect of Doppler shifts in relation to echo flow. Doppler shifts, a shift in frequency that is relative to movement of the listener and sound source, may provide information to echolocating bats navigating the echo flow corridor. The FM echolocation calls of the big brown bat, which is the subject of both studies presented in Chapters 2 and 3, are largely Doppler tolerant (Altes and Titlebaum, 1970). By contrast, the acoustic features of CF signals are well suited to carry Doppler flow information from objects of the bat's environment, as frequency shifts are proportional to the relative velocity of the bat and objects along its trajectory (Mueller and Schnitzler, 1999). The Taiwanese leaf-nosed bat may thus be able to utilize specific information embedded in

Doppler flow to guide its navigation. It would be interesting to learn more about this species' adaptation to echo flow. I suggest that a larger corridor, which accommodates the bat's size, and more individuals would produce a cleaner data set, from which conclusive results can be drawn with respect to the flight adaptation of *H. a. terasensis*, and its use of Doppler flow in flight.

Making use of the long echolocation sounds of CF bats, Carmen and Hallam (2004) have demonstrated that Doppler information can be used to implement basic obstacle-avoidance navigation. Peremans and Steckel (2014) have subsequently succeeded in creating a basic robotic-guided motion control system using Doppler flow patterns. More recently, a few research groups have used principles of echolocation and acoustic flow to develop sonar devices that navigate through acoustically cluttered environments (e.g. Baker et al., 2014; Smith et al., 2013) , with the goal of showing that it is possible to extract sufficient information from echo signals to guide an autonomous agent (Steckel and Peremans, 2017). For example, Steckel and Peremans (2017) show simulated and empirical data from a moving platform robot equipped with a sonar device set to navigate through a cluttered environment using different strategies, including "acoustic flow corridor-following." This strategy describes a sophisticated use of separate echoes from objects straight ahead that steer the robot left- or rightward. In the simulations, the device was programmed to navigate through a 2 m wide corridor containing 10 randomly distributed objects. One hundred runs of the simulation, and later testing of the robot in the actual environment, show reliable navigation without collision, even when an object is placed in the middle of the navigational path. Overall, using acoustic flow in the

calculations to guide robot navigation enabled straightening out and correcting erroneous navigation.

Overall, the results of our echo flow studies (Chapters 2, 3 and 4) advance the knowledge of biological sonar processing of complex soundscapes, and hold importance to research on sensory representation and action selection in freely moving animals that rely on other sensory modalities. I conjecture that our findings inspire research on real-time sonar processing incorporated in autonomous vehicles for efficient navigation, and that such sonar navigation systems can also inform our understanding of bat navigation. For example, it would be interesting to test the behavior of sonar-based navigational systems in environments that mirror our echo flow corridor. Steckel and Peremans (2017) programmed several different navigational strategies into their robot before it navigated a complex environment using acoustics. We could learn much about navigation using acoustics induced by echo flow by letting such robotic systems navigate an environment like our echo flow corridor using either FM or CF signals, while programmed to use the same strategies. Whichever combination of strategies and acoustic signal most closely reproduces behavior seen in the echolocating bat, may help inform our understanding of bat navigation in such environments, and fuel ideas for new experiments.

While bats may navigate along forest edges and within clutter, they also forage among other conspecifics, which exposes them to acoustic interference from conspecific sonar sounds. In fact, bats are social animals and commonly exit their caves to hunt in large groups in open and also cluttered environments. In such situations, echo signals can be complex, meaning the sound wave that contains information of the target object often

is merged with sound waves of other object's echoes or other bats' sonar emissions. For example, when a bat is approaching a detected prey item while foraging in a cluttered environment among other conspecifics, it its acoustic input will be composed of a linear combination of its own vocalization, echoes from its own calls, several other bats' calls and corresponding echoes at different amplitudes, as well as any environmental noises that might occur. With this complex sound wave as auditory input, the bat has to separate out the echo that belong to its target of interest, in order to not lose the moving prey. While some noises may be ignored more easily due to off-axis lowpass filtering (Warnecke et al., 2014), others may originate from locations close to the prey and contain frequencies that overlap with those of the bat's vocalization, complicating sound segregation. Despite these challenges, studies and observations have shown that bats have no problem foraging among one another, navigating through tight spaces, and foraging in densely-cluttered vegetation. Previous research has given some clues as to what mechanisms may allow the bat to sort its own calls from that of a nearby bat (e.g. Obrist 1995; Chiu et al., 2008, 2009), and aid in the analysis of complex spectro-temporal echo signals (Simmons et al., 1999; Bates et al., 2011).

One major component that previous research reported as a mechanism to avoid acoustic interference, or eavesdrop on another bat, is "silent behavior" (Chiu et al., 2008). Chiu and colleagues discovered that paired bats competing for a single prey item will sometimes go silent, that is cease to echolocate for at least 200 ms. They report that this behavior is most often observed by the following bat when two bats are in following flight and at inter-bat distances smaller than 1 m. Interestingly, the bat that was last silent was also the one that caught the prey, hypothesizing that the following, last silent bat may

have been listening to echoes from the prey in preparation to capture it before the leading bat could. Further, the probability of silent behavior increases with increasing baseline call similarity, meaning that bats with similar call features are more likely to exhibit silent behavior.

What factors drive silent behavior and what is its function? While analyses by Chiu et al. (2008) helped explain when silent behavior was most likely to occur, they did not conclusively explain what the function of silent behavior may be. For example, in their data set it was the following bat that ceased echolocating, and it was the bat that last exhibited silent behavior which caught the prey. We can thus hypothesize that one function of silence is "stealth behavior", or eavesdropping, to increase chances of prey capture. This hypothesis is supported by the fact that the last silent bat caught the prey more often. In this scenario, the following bat is not emitting echolocation calls, so the leading bat will not be getting any acoustic cues as to the other bat's current whereabouts: there are no conspecific calls, and the sonar beam of the leading bat will not ensonify object's behind itself, so that there cannot be any echoes of(f) the following, silent bat, giving away its relative location. This suggests that the following bat, presumably listening to calls and echoes of the leading bat, must detect, for example, buzzing behavior of the leading bat, increase its flight speed to fly ahead and capture the prey. Such behavior would qualify being labeled "stealth" or "eavesdropping", so as to not warn the leading bat of one's own location until the prey is close enough. This situation presumes that the silent bat is listening to the leading bat's echolocation calls and echoes returning from the environment, and either uses the absolute information for its flight guidance, or is able to account for the fact that it is several centimeters behind the leading

bat which emitted the call, using relative information for flight guidance. Note that the following bat's soundscape is different from that of the leading bat, not only because the other bat's call structure might differ from its own, but also because the spatial locations of the two bats differ.

While it is possible that silence could be employed for such "stealth behavior", another function of silent behavior could be to prevent acoustic interference of the soundscape by reducing the amount of echoes and reverberation caused by bat sonar emissions. This hypothesis stems from general challenges that arise as more sound sources are introduced. In this context, since silent behavior is most often seen in bats with similar calls structures, calls emitted and echoes received by the two bats might be more difficult to separate perceptually. Silent behavior may thus reduce acoustic interference.

The observation of silent behavior as a mechanism to reduce acoustic interference or eavesdrop during foraging tasks in open flight spaces (Chiu et al., 2008) motivated the research in **Chapter 5**, in which I investigated whether silent behavior persisted in cluttered environments. Bats navigated both empty and cluttered flight spaces in pairs while they competed for a single prey item. We hypothesized significant amounts of silent behavior across different bat pairs in the empty environment, as had been shown by Chiu et al. (2008). By contrast, we hypothesized little to no silent behavior in the forest, as bats needed to monitor their immediate surroundings more closely. While no silent behavior was detected in cluttered environments, the data also did not replicate Chiu et al.'s reports of silent behavior in the open flight space. This surprising result spurred

further questions about silent behavior, including what motivates periods of silent behavior, and what is its function? The data in Chapter 5 suggested that gender and flight configurations could be related to the prevalence of silent behavior. In an effort to understand how the interplay of environment, prey availability and gender may relate to silent behavior, **Chapter 6** presented data from a large-scale study which manipulated all of these components. Single and paired bats of all gender combinations flew in empty and cluttered flight rooms, while they were either just navigating the space or foraging. We focused the analysis of the data set on the condition exposing pairs of bats to an open flight space while they competed for a single prey item, in order to replicate silent behavior reports of Chiu et al. (2008), and understand the influence of different gender combinations on the prevalence of silence. We found only very small amounts of silence across bat pairs, which did not match the expectations set by Chiu et al. (2008). This suggests that Chiu et al. (2008)'s work might represent an isolated incident, or be subject to technological shortcomings of experimental equipment at the time.

The presence of silence would presume that the silent bat might listen to the leading bat's echolocation calls and echoes to navigate. However, as we found only small amounts of silent behavior, I hypothesize that echolocating bats may need continuously updated information from their own vocalizations to effectively navigate and forage. We found that in the empty flight room – single prey conditions pulse intervals lasting longer than 200 ms averaged about 250 ms in single bat flights, and 350 ms in paired bat flights. This suggests that single bats may need to sample their environment at least every 250 ms to navigate successfully, while paired bats may be able to make use of the other bat's echolocation calls, and reduce their minimum necessary sampling of the environment to

350 ms intervals. These behaviors also point to small amounts of silent behavior as being a way to reduce acoustic interference, rather than eavesdrop intentionally. However, whether silent behavior as such is a separate strategy rather than just a "long pulse interval" that is utilized when other signals are present so as to not "fly blindly" (pun intended), is still a matter of debate.

In summary, the work in this thesis has elucidated behavioral adaptations to different echo flow patterns, and informed our understanding of the impact of object location on flight path planning in bats. The results can guide hypotheses and experimental designs in future research to evaluate behavioral and neural modulations to spatio-temporal changes in the environment, and inspire questions about the neural processing of acoustic stimuli comprising the bat's natural soundscape. Further, we have gained new insight into the importance of silent behavior, which has long been accepted as a common principle in multiple bat foraging situations. The results of our studies lead to new questions about its significance and re-ignite research into mechanisms bats might employ to avoid acoustic interference.

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# Curriculum vitae

Michaela Warnecke was born in Münster, Germany, in 1988. She spent her undergraduate years at the University of Osnabrück, Germany, and received a B.Sc. in Cognitive Science. During her time as an undergraduate, she spent one year in the laboratory of James A. Simmons at Brown University, RI, and studied changes in bat perception using 2 AFC tasks. After completing her Bachelor's degree, she returned to Brown University as the lab manager in Andrea M. Simmons' FrogLab, and James A. Simmons' BatLab. While at Brown University, Michaela worked on the lateral line system of the bullfrog, and investigated the perception of target and clutter objects in bat biosonar.

Michaela started graduate school in the Department of Psychological and Brain Sciences at the Johns Hopkins University in Baltimore, MD, in the Fall of 2013. She worked with Cynthia F. Moss, investigating how freely-flying echolocating bats analyze their acoustic scene. In 2014, she received the ERP Graduate Fellowship from the Studienstiftung in Germany, which supported her work for the following 2 years. She received her Master's degree in 2015. During her time at JHU, she TA-ed for several classes, and taught her own class, "Good Vibrations", in the fall of 2017. From 2014 to 2018, she was also the animal bioacoustics student representative for the Acoustical Society of America, and in that capacity launched several outreach and networking initiatives.